# FERAL AFRICANIZED HONEY BEE ECOLOGY IN A COASTAL PRAIRIE LANDSCAPE

A Dissertation

by

KRISTEN ANNE BAUM

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2003

Major Subject: Entomology

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Approved as to style and content by:

| | |
|---|---|
| Robert N. Coulson<br>(Co-Chair of Committee) | William L. Rubink<br>(Co-Chair of Committee) |
| William E. Grant<br>(Member) | S. Bradleigh Vinson<br>(Member) |
| X. Ben Wu<br>(Member) | Kevin M. Heinz<br>(Head of Department) |

May 2003

Major Subject: Entomology

# ABSTRACT

Feral Africanized Honey Bee Ecology in a Coastal Prairie Landscape. (May 2003)

Kristen Anne Baum, B.S., The College of William and Mary;

M.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Robert N. Coulson
Dr. William L. Rubink

Honey bees, *Apis mellifera*, play an important role in many ecosystems, pollinating a wide variety of native, agricultural, and exotic plants. The recent decline in the number of feral and managed honey bee colonies in North America, as well as the arrival of Africanized honey bees, have caused concern about adequate pollination for agricultural crops and natural plant communities. However, little is known about feral colonies, and the feral population is the source for Africanized honey bees as they spread and infiltrate managed populations.

The goal of my dissertation was to examine the ecology of feral honey bee colonies, adding the spatial context necessary to understand the population ecology and patterns of resource use by feral honey bees on the Welder Wildlife Refuge. I defined the functional heterogeneity of feral honey bee habitat by identifying the suitability of different habitats for feral colonies based on the distribution and abundance of important resources (cavities, nectar, and pollen). I evaluated the distribution and abundance of feral colonies by examining nest site characteristics, population trends, and spatial and

temporal patterns in cavity use. Lastly, I examined resource use by evaluating patterns in pollen collection and identifying where and when honey bees searched for resources.

Overall, the Welder Wildlife Refuge provided excellent habitat for feral honey bees, supporting a high density of feral colonies. The dense live oak habitat was the best overall source for cavities, nectar, and pollen. Nectar and pollen were abundant throughout the year, with the exception of December and January, when a large number of honey bees searched for resources. Cavities did not appear to vary in their suitability for feral colonies based on measured structural and environmental attributes, since no cavity attributes were correlated with indices of cavity quality. However, the cavity quality indices varied between cavities, suggesting some cavities were more suitable for feral honey bees than others. Colonies were aggregated within the study area, probably due to the distribution of resources. The invasion of Africanized honey bees appeared to fragment the existing European population, with Africanized colonies aggregated in distribution and European colonies random in distribution.

# DEDICATION

This dissertation is dedicated to Blannie who taught me what is really important, and to my mom, dad, and sister who gave me the courage to never give up.

# ACKNOWLEDGEMENTS

I thank my advisory committee: Drs. Bob Coulson, Bill Rubink, Bill Grant, Ben Wu, and Brad Vinson, for their help in the development and completion of this research project. Special thanks to Dr. Bob Coulson and Dr. Bill Rubink for serving as my committee co-chairs and participating in all aspects of this project.

This research would not have been possible without the involvement and support of numerous people. Dan Saenz helped develop many of the ideas and assisted with the collection of field data. Dr. Tanya Pankiw provided insightful discussions on honey bee biology, as well as guidance throughout my graduate career. Alice Pinto joined me on my journey as a Ph.D. student, and provided some of the data used in this dissertation. Dr. Doug Wunneburger developed the mosaic of the Welder Wildlife Refuge and assisted in GIS related components of this project. Lisa Lavold and Dawn Marshall processed and identified many of the pollen samples, and Dr. Vaughn Bryant provided valuable input on pollen data interpretation. Dr. Lynn Drawe, Dr. Terry Blankenship, and Dr. Selma Glasscock provided data from the Welder Wildlife Refuge and provided support during my time in the field. Jim McCormick, Art Cavazos, and Roy Medrano provided additional help in the field. Audrey Bunting, Maria Tchakerian, and Rebecca Meegan provided technical and moral support in College Station. Dr. Bill Wilson shared his keen interest in honey bees and many references. Lastly, I would like to thank my friends and family for their constant support throughout my graduate studies.

Funding for this project was provided by the Welder Wildlife Foundation, the USDA/ARS/Beneficial Insects Research Unit, and the Texas Legislative Initiative: Protection and Management of Honey Bees - Pollinators of Agricultural Crops, Orchards, and Natural Landscapes.

# TABLE OF CONTENTS

Page

ABSTRACT ..... iii

DEDICATION ..... v

ACKNOWLEDGEMENTS ..... vi

TABLE OF CONTENTS ..... viii

LIST OF FIGURES ..... x

LIST OF TABLES ..... xiii

CHAPTER

I INTRODUCTION ..... 1

Background ..... 2
Study system ..... 5

II HABITAT ASSOCIATIONS ..... 7

Introduction ..... 7
Methods ..... 9
Spatial database development ..... 9
Landscape classification and quantification ..... 10
Nectar classification ..... 18
Pollen classification ..... 22
Cavity classification ..... 24
Combined classification ..... 25
Results ..... 28
Landscape classification and quantification ..... 28
Nectar classification ..... 28
Pollen classification ..... 35
Cavity classification ..... 49
Combined classification ..... 61
Discussion ..... 61

III DISTRIBUTION AND ABUNDANCE ..... 74

CHAPTER Page

Introduction ..... 74
Methods ..... 76
Study site description ..... 76
Colony density ..... 77
Cavity attributes ..... 78
Spatial and temporal patterns ..... 80
Spatial and temporal patterns of Africanized and European colonies ..... 81
Results ..... 82
Colony density ..... 82
Cavity attributes ..... 82
Spatial and temporal patterns ..... 85
Spatial and temporal patterns of Africanized and European colonies ..... 92
Discussion ..... 92
Colony density ..... 92
Cavity attributes ..... 106
Spatial and temporal patterns ..... 110
Spatial and temporal patterns of Africanized and European colonies ..... 114
Conclusions ..... 115

IV RESOURCE USE – POLLEN ..... 117

Introduction ..... 117
Methods ..... 118
Results ..... 125
Discussion ..... 140

V RESOURCE USE – AERIAL PITFALL TRAPS ..... 150

Introduction ..... 150
Methods ..... 151
Results ..... 155
Discussion ..... 162

VI CONCLUSIONS ..... 166

LITERATURE CITED ..... 170

VITA……. ..... 182

# LIST OF FIGURES

Page

Figure 1. Modifications for minimum patch size requirements. ..... 16

Figure 2. The NetWeaver™ dependency network for the knowledge base on feral honey bee behavior in coastal prairie landscapes. ..... 27

Figure 3. Vegetation community classification at the 5000 m2 minimum patch size. ..... 30

Figure 4. Nectar classification for January. ..... 37

Figure 5. Nectar classification for February. ..... 38

Figure 6. Nectar classification for March through September. ..... 39

Figure 7. Nectar classification for October. ..... 40

Figure 8. Nectar classification for November. ..... 41

Figure 9. Nectar classification for December. ..... 42

Figure 10. Pollen classification for January. ..... 51

Figure 11. Pollen classification for February. ..... 52

Figure 12. Pollen classification for March and April. ..... 53

Figure 13. Pollen classification for May through September. ..... 54

Figure 14. Pollen classification for October. ..... 55

Figure 15. Pollen classification for November. ..... 56

Figure 16. Pollen classification for December. ..... 57

Figure 17. Overall cavity classification. ..... 60

Figure 18. Overall nectar classification. ..... 62

Page

Figure 19. Overall pollen classification. ..... 63

Figure 20. Combined classification. ..... 64

Figure 21. Vegetation classification for San Patricio County, Texas and the Welder Wildlife Refuge from McMahan et al. (1984). ..... 71

Figure 22. Vegetation classification for counties in the Texas coastal bend from McMahan et al. (1984). ..... 72

Figure 23. Tree species containing cavities used by feral honey bee colonies on the Welder Wildlife Refuge. ..... 84

Figure 24. Entrance orientation of cavities used by feral honey bee colonies on the Welder Wildlife Refuge. ..... 87

Figure 25. Location of all identified cavities within each habitat type on the Welder Wildlife Refuge. ..... 88

Figure 26. Frequency distribution of the time occupied index for all cavities identified by 1995 on the Welder Wildlife Refuge. ..... 89

Figure 27. Frequency distribution of the turnover index for all cavities identified by 1995 on the Welder Wildlife Refuge. ..... 90

Figure 28. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1993. ..... 94

Figure 29. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1994. ..... 95

Figure 30. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1995. ..... 96

Figure 31. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1996. ..... 97

Figure 32. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1997. ..... 98

Figure 33. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1998. ..... 99

Page

Figure 34. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1999. ....100

Figure 35. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 2000. ....101

Figure 36. Vegetation classification for the Texas coastal bend from McMahan et al. (1984). ....112

Figure 37. Cavity and pollen trap locations. ....119

Figure 38. Dead bee trap modified to hold a removable pollen screen. ....120

Figure 39. A landscape classification based on vegetation communities showing the spatial distribution of habitats in the study area. ....124

Figure 40. Light micrographs of pollen types collected by honey bees. ....128

Figure 41. Percent of entomophilous, anemophilous, both, and unidentified pollen types collected each sampling period. ....141

Figure 42. Percent of pollen types from herbs, trees, shrubs, combination, and unidentified pollen sources collected each sampling period. ....142

Figure 43. Percent of pollen types from nectariferous, non-nectariferous, and unidentified pollen sources collected each sampling period. ....143

Figure 44. Aerial pitfall trap design. ....153

Figure 45. Location of aerial pitfall traps within each habitat type on the Welder Wildlife Refuge. ....154

Figure 46. Mean number of honey bees collected per trap in relation to nectar and pollen availability for each sampling period. ....158

Figure 47. Mean number of honey bees collected per trap for each habitat type and sampling period. ....161

# LIST OF TABLES

Page

Table 1. Landscape classification criteria. ..... 11

Table 2. Values assigned for nectar importance, pollen importance, plant abundance, and plant growth form. ..... 19

Table 3. Selected class metrics for the 5000 $m^2$ minimum patch size classification. ..... 29

Table 4. Important nectar sources by habitat type and month. ..... 31

Table 5. Nectar availability between months and habitat types. ..... 34

Table 6. Nectar availability between habitat types for each month. ..... 36

Table 7. Conservative estimates of the kg of sugar produced in the study site, as well as the kg of sugar produced within the cumulative potential foraging range of all the colonies in the study site. ..... 43

Table 8. Important pollen sources by habitat type and month. ..... 44

Table 9. Pollen availability between months and habitat types. ..... 48

Table 10. Pollen availability between habitat types for each month. ..... 50

Table 11. Conservative estimates of the kg of pollen produced in the study site, as well as the kg of pollen produced within the cumulative potential foraging range of all the colonies in the study site. ..... 58

Table 12. Overall classifications for nectar, pollen, and cavity availability and the combined classification for resource availability by habitat type. ..... 59

Table 13. Colony density for each year based on a 6.25 $km^2$ study area. ..... 83

Table 14. Descriptive statistics for the measured structural and environmental attributes of cavities occupied by feral honey bee colonies on the Welder Wildlife Refuge. ..... 86

Page

Table 15. Results from Spearman correlation tests comparing the structural and environmental cavity attributes and time occupied and turnover indices and Mann-Whitney *U* tests comparing cavities used only by Africanized or European colonies. ..... 91

Table 16. Spatial and temporal patterns identified by a nearest neighbor analysis of cavities used by feral honey bee colonies on the Welder Wildlife Refuge. ..... 93

Table 17. Spatial and temporal patterns identified by a nearest neighbor analysis of cavities occupied by European and Africanized colonies on the Welder Wildlife Refuge. ..... 102

Table 18. Feral colony densities reported in the literature and this study. ..... 104

Table 19. Cavity attributes compared between this study and similar data reported in the literature. ..... 107

Table 20. Identified pollen types by family. ..... 126

Table 21. Shaded areas represent predominant pollen types and numbers represent the percentage of a cumulatively important pollen type for a given time period. ..... 132

Table 22. Means, standard deviations, and p-values from a Wilcoxon signed rank test comparing the overlap of collected pollen types between pairs of colonies and sampling periods. ..... 134

Table 23. Means, standard deviations, rho values, and p-values from a Spearman rank test for correlation between pollen overlap and distance between colonies ..... 135

Table. 24. The abundance of predominant and cumulatively important pollen types in different habitat types within the study area based on data from point frame transects provided in the Welder plant list ..... 137

Table 25. Predominant and cumulatively important pollen types with overestimated and underestimated contributions based on volume. ..... 138

Table 26. Means, standard deviations, Z-values, and p-values for Wilcoxon signed rank tests comparing the number of honey bees collected per trap between sampling periods. ..... 156

Page

Table 27. The results from Kruskal-Wallis and Mann-Whitney *U* tests comparing the number of honey bees collected per trap between habitat types, where B = brushland, G = grassland, O = live oak, and W = woodland.....159

# CHAPTER I

# INTRODUCTION

Honey bees, *Apis mellifera*, play an important role in many ecosystems, pollinating a wide variety of native, agricultural, and exotic plants. The recent decline in the number of feral and managed honey bee colonies in North America has caused concern about adequate pollination for agricultural crops and natural plant communities (Buchmann and Nabhan 1996, Ingram et al. 1996, Allen-Wardell et al. 1998). Morse and Calderone (2000) estimated the value of pollination services provided by honey bees in the United States at 14.6 billion dollars annually.

To date, studies of feral honey bees have identified and described the locations of colonies (Taber 1979, Schneider and Blyther 1988, Morse et al. 1990, Ratnieks et al. 1991, Oldroyd et al. 1995, McNally and Schneider 1996). However, these data have not been related to the spatial configuration of resources important for the survival, growth, and reproduction of honey bee colonies. Since the immigration of Africanized honey bees in the United States, understanding the ecology of feral colonies has become very important, since the feral population provides the source for Africanized honey bees as they spread and infiltrate managed populations. Therefore, more information on the ecology of feral colonies is needed to address important issues related to pollination and the dispersal of Africanized honey bees.

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This dissertation follows the style and format of Ecology.

The goal of my dissertation is to examine the ecology of feral honey bee colonies, adding the spatial and temporal context necessary to understand the population ecology and patterns of resource use by feral honey bees on the Welder Wildlife Refuge. Data on feral honey bee colonies have been collected on the Welder Wildlife Refuge for more than ten years (unpublished data, W. L. Rubink). Based on this background research, I will address the following objectives:

1) identify habitat associations of feral honey bee colonies,
2) evaluate changes in the distribution and abundance of feral honey bee colonies, and
3) examine resource use by feral honey bee colonies.

More specifically, I will define the functional heterogeneity of feral honey bee habitat by identifying the suitability of different habitats for feral colonies based on the distribution and abundance of important resources (cavities, nectar, and pollen). I will evaluate the distribution and abundance of feral colonies by examining nest site characteristics, population trends, and spatial and temporal patterns in cavity use. Lastly, I will examine resource use by evaluating patterns in pollen collection and identifying where and when honey bees search for resources.

## BACKGROUND

Honey bees are native to the Old World and can be separated into races based on geographic region (Ruttner 1988). For this study, the distinction of interest is between

European and Africanized honey bees. Settlers introduced honey bees of European origin into eastern North America in the early to mid 1600's (Sheppard 1989a, 1989b). African honey bees were first brought to Brazil in 1956 to develop a hybrid more suitable for honey production in Brazil's tropical climate. The colonies swarmed and quickly became established in the local feral population, rapidly spreading through South and Central America and Mexico and reaching Texas in 1990 (Hunter et al. 1993, Rubink et al. 1996). Whether these bees should be called Africanized (Rinderer 1986) or African (Taylor 1988, Hall and Muralidharan 1989, Smith et al. 1989, Hall 1990) is controversial. The debate revolves around whether these bees show intermediate characteristics between European and African bees, or whether they show little or no introgression with European bees. It is also interesting to note that the European population includes not only introductions of subspecies from Europe, but also from Egypt (*A. m. lamarckii*), North Africa (*A. m. intermissa*), and the near East (*A. m. caucasica*, *A.m. cypria*, and *A. m. syriaca*) (Sheppard 1989a, 1989b). However, for simplicity I will refer to the bees introduced into Brazil as Africanized and the original resident population in North America as European.

Behavioral differences related to the predictability of resources exist between Africanized and European honey bees. Africanized honey bees are characterized by accentuated defensive behavior, higher rates of brood production, and more frequent swarming and absconding (Winston 1991b). These attributes are beneficial to survival in an unpredictable environment. The defensive behavior of Africanized bees involves faster response times, increased numbers of alerted bees, and more stinging activity

compared to European bees (Collins et al. 1982). Brood production is faster in Africanized bees, due to shorter development times and higher rates of brood rearing (Winston 1991b). Swarming, or colony fission, is the means by which honey bee colonies reproduce. A portion of the established colony leaves, forming a new founder colony. Initial swarms, referred to as prime swarms, typically contain most of the workers and the old queen of the established colony. Prime swarms are often followed by smaller afterswarms (Winston 1991a). Absconding refers to the abandonment of a nest site by the entire colony, typically caused by a disturbance or seasonal change in resource availability. Absconding and swarming are more common in bees of tropical origin, such as African bees (Winston 1991a). Overall, these traits are incompatible with current beekeeping practices, making managed colonies difficult to handle, impacting contracts for crop pollination, and posing potential hazards to human and livestock health (Spivak et al. 1991).

In addition to the characteristics described above, foraging differences have been identified between European and Africanized honey bees. Several studies have reported that Africanized colonies contain a larger proportion of pollen foragers and store larger amounts of pollen than European colonies (Danka et al. 1987, Pesante et al. 1987, Schneider and Hall 1997). However, other studies have found that Africanized colonies forage less for pollen than European colonies (Pankiw and Rubink 2002) and show lower rates of recruitment to pollen sources (Danka et al. 1988). Africanized honey bees also have lower response thresholds for sucrose, responding to lower concentrations than European honey bees (Pankiw and Rubink 2002), and typically forage shorter distances compared to European

honey bees or hybrids (Danka et al. 1993, Schneider and Hall 1997). These differences have important implications for the pollination efficacy of European and Africanized honey bees.

## STUDY SYSTEM

The Welder Wildlife Refuge is 7800 acres of coastal prairie habitat located in San Patricio County, about 35 miles north of Corpus Christi, Texas. The refuge is actively managed for cattle ranching and supports a wide array of wildlife research. Dr. William L. Rubink has monitored feral honey bee colonies on the Welder Wildlife Refuge for more than ten years. To date, 109 cavities have been located and are surveyed yearly for the presence or absence of feral honey bees.

Africanized honey bees were first recorded in San Patricio County in 1992 and on the Welder Wildlife Refuge in 1993 (unpublished data, W. L. Rubink). Based on an analysis of mitochondrial DNA, approximately 20 percent of the colonies sampled in 2000 had non-African mitochondrial DNA, while the remaining 80 percent had African mitochondrial DNA (unpublished data, M. A. Pinto, W. L. Rubink, J. S. Johnston, and R. N. Coulson). Mitochondrial DNA is maternally inherited and does not recombine during sexual reproduction, passing directly from queen to offspring. Therefore, most of the colonies on the Welder Wildlife Refuge are probably Africanized, based on the high probability that European queens have mated with Africanized drones. The overall abundance of Africanized drones should be at least three times greater than European drones, since there are three times as many African matriline colonies and Africanized honey bees devote more space to drone comb than European honey bees (Winston

1988). Africanized drones also migrate into European colonies, decreasing European drone production and further enhancing Africanized drone production (Rinderer et al. 1985). Queens typically mate with 7 to 17 drones (Taber and Wendel 1958, Adams et al. 1977), so the probability of Africanization is very high. Additional support is provided by Clarke et al. (2002), who found substantial paternal gene flow from feral Africanized colonies into the European population three years after Africanization in the Yucatan Peninsula, Mexico. Therefore, I will identify habitat associations, evaluate changes in distribution and abundance, and examine resource use by honey bee colonies in a system of feral Africanized honey bees.

This dissertation is written in chapter format, with a separate chapter(s) for each objective. After the general introduction (Chapter 1), the second chapter describes the development of a spatial database for the Welder Wildlife Refuge and a landscape classification for the study area based on vegetation communities. The landscape classification is used to evaluate the suitability of different habitats for feral colonies based on the distribution and abundance of important resources (cavities, nectar, and pollen), and the functional heterogeneity of feral honey bee habitat is defined using a knowledge engineering approach. In the third chapter, I evaluate the distribution and abundance of feral colonies by examining nest site characteristics, population trends, and spatial and temporal patterns in cavity use. The fourth and fifth chapters examine resource use by evaluating patterns in pollen collection and identifying where and when honey bees search for resources. The sixth chapter provides an overall summary of the conclusions resulting from each objective.

# CHAPTER II

# HABITAT ASSOCIATIONS

## INTRODUCTION

Landscapes consist of a variety of habitats that vary in suitability for different animals. Suitability often differs depending on the species, activity (nesting, foraging, etc.), life cycle stage, or time of year (Orians and Wittenberger 1991, Morris 1992). For example, a habitat with abundant pollen and nectar sources important for foraging may not contain cavities necessary for nesting. Therefore, a landscape consists of a mosaic of habitats varying in suitability based on a number of different factors that often can be described in terms of environmental resources. The suitability of a given habitat type for a particular organism can be combined for all resources important to that organism to obtain an overall suitability ranking for that habitat. Then, rankings can be compared between habitats to evaluate their value to a particular species (Gerrard et al. 2001) or between species to evaluate interspecific interactions within a landscape (Coulson et al. 1999). This approach forms the basis for understanding functional heterogeneity, how an organism perceives and responds to different landscape elements (Forman 1995). Thus, the overall goal of this study was to define the functional heterogeneity of feral honey bee habitat based on the distribution and abundance of resources important for feral honey bee colonies. More specifically, I will develop a methodology for classifying landscapes using a rule-based approach. I will evaluate how feral honey bee colonies use coastal prairie habitat on the Welder Wildlife Refuge. Lastly, I will

extrapolate patterns of resource use from the study area to the county and ecoregion scales.

Important resources for feral honey bees include cavities, pollen, nectar, water, and propolis. Cavities vary in their suitability for feral colonies based on structural characteristics, as well as local environmental conditions (Avitabile et al. 1978, Rinderer et al. 1982, Schneider and Blyther 1988, Gambino et al. 1990, Ratnieks et al. 1991, Morse et al. 1993, Schmidt and Hurley 1995, McNally and Schneider 1996). Pollen, nectar, and water vary in quality depending on their distribution, abundance, handling time, and composition (Vivino and Palmer 1944, Percival 1961, Lüttge 1977, Roulston and Cane 2000, Roulston et al. 2000). Propolis is a resinous plant-derived material used by honey bees for a variety of purposes, including to fill cracks and crevices in the hive (Schmidt and Buchmann 1999). The composition of propolis varies depending on the source and may influence the usefulness of the material in the hive (Schmidt and Buchmann 1999).

Of these important resources identified for feral honey bee colonies, pollen and cavities are the most easily studied in a spatial context. Pollen can be collected from foraging honey bees returning to the colonies, identified, and related to the spatial location of the source plants. An alternative method would involve observing recruitment dances within the hive, but this is not feasible inside natural tree cavities. Nectar sources are more difficult to study under natural conditions, since nectar cannot be as easily collected from returning foragers or the source as easily identified (although the pollen grains found in nectar can be used to identify nectar sources after considering

the filtering abilities of a honey bee's proventriculus in relation to the size of the pollen grains (Maurizio 1951, Jones and Bryant 1996)). However, inferences can be made about the nectar sources used by feral colonies based on pollen sources that also provide nectar, frequently used honey plants, and knowledge about plant communities and flowering phenology in the study area. The location of cavities used by feral colonies can be recorded, and detailed measurements obtained for each. Potential water sources can also be identified and their locations recorded.

I collected data from feral honey bee colonies on the Welder Wildlife Refuge, including information about pollen and cavity use. This information was used to develop a series of classifications for the study site, including classifications for nectar, pollen, and cavity availability. These classifications were examined to identify the importance of different areas on the Welder Wildlife Refuge for feral honey bees.

## METHODS

### Spatial database development

A spatial database of the Welder Wildlife Refuge was developed as a joint project between Texas A&M University, the USDA/ARS Beneficial Insects Research Unit, and the Welder Wildlife Refuge. Approximately 70 high resolution, color infrared images of the Welder Wildlife Refuge at 5500 feet above ground level were taken by the USDA/ARS Remote Sensing Unit (M. René Davis, pilot) during October 1999. A large format (23 cm x 23 cm) mapping camera with a 305 mm lens was used with a fixed-wing aircraft for the aerial photography. Each photograph covered approximately 1265

m of lateral distance on the ground with a scale of about 1:5500. There was approximately 60 % overlap between photographs in the same flight line, and approximately 30 % overlap between flight lines. In order to develop a digital database of the Welder Wildlife Refuge, the color positive transparencies were scanned at 600 dpi using an EPSON® Expression® 836XL scanner equipped with a transparency unit. The images were registered using ground control points. When possible, harvester ant (*Pogonomyrmex* spp.) mounds, which appeared as white dots of bare ground with a two to four m diameter on the aerial photographs, served as ground control points. The coordinates for each ground control point were recorded to a submeter accuracy using a Trimble GPS Pathfinder™ receiver and TSC1™ Asset Surveyor™ data logger. Each image was geographically registered using Erdas Imagine®. After georeferencing, the images were transformed using a polynomial transformation and resampled to a 0.25 m resolution. Dr. Doug Wunneburger (GeoInformatics Studio, College of Architecture, Texas A&M University) developed the mosaic using Intergraph® IRASC.

Landscape classification and quantification

I developed a landscape classification of the vegetation communities on the Welder Wildlife Refuge using the mosaic described above. A 25 m grid was placed over the mosaic of the study area and each grid cell was classified in ArcView® GIS 3.2 according to a set of rules about landscape cover (Table 1). If multiple coverage criteria were met, then priority rankings were used to select the plant community with the highest ranking (1=highest, 10=lowest). For example, if a cell was 50 % grassland and

Table 1. Landscape classification criteria. Coverage refers to the amount of the listed plant community that must be present for a grid cell to be classified as that habitat type. Priority rankings were from 1 (highest) to 10 (lowest) and similarity groupings were given priority from left (highest) to right (lowest) when multiple groupings were listed for a plant community.

| plant community | coverage | priority | similarity groupings | | |
|---|---|---|---|---|---|
| aquatic plants (A) | >50% | 1 | WA | | |
| brushland (dense) (B-D) | >75% | 5 | B-O | | |
| brushland (open) (B-O) | 25-75% | 6 | B-D | B-G | |
| brushland-grassland (B-G) | >25% (grass) | 7 | B-O | G | WO |
| grassland (G) | >25% | 8 | LO-O | B-G | WO |
| live oak (dense) (LO-D) | >75% | 2 | LO-O | | |
| live oak (open) (LO-O) | 25-75% | 3 | LO-D | LO-G | |
| disturbed (D) | >50% | 9 | G, B-G | | |
| water (WA) | >50% | 10 | A | | |
| woodland (WO) | >75% | 4 | G, B-G | | |

50 % open live oak, that cell was classified as open live oak (Table 1). Priorities were selected to preserve uncommon plant communities (aquatic plants), eliminate communities without resources (disturbed) or with unlimited resources (water), and emphasize potentially limiting resources (cavities).

The brushland community occurred on clay and clay loam soils (Drawe et al. 1978). Nomenclature for describing the plant communities follows Jones et al. (1997). Dominant woody species included blackbrush acacia (*Acacia rigidula* G. Bentham), agarito (*Berberis trifoliata* M. Moricand), and honey mesquite (*Prosopis glandulosa* J. Torrey var. *glandulosa*). Huisache (*Acacia minuata* (M. E. Jones) P. de Beauchamp subsp. *minuata*), woolybucket bumelia (*Sideroxylon lanuginosum* A. Michaux), granjeno (*Celtis pallida* J. Torrey), Texas persimmon (*Diospyros texana* G. Scheele), and lime pricklyash (*Zanthoxylum fagara* (C. Linnaeus) C. Sargent) were also present. Western ragweed (*Ambrosia psilostachya* A. P. de Candolle), spiny aster (*Chloracantha spinosa* (G. Bentham) G. Nesom var. *spinosa*), and upright prairie coneflower (*Ratibida columnifera* (T. Nuttall) E. Wooton and P. Standley) comprised the dominant forbs. Important grasses included knotroot bristlegrass (*Setaria parviflora* (J. Poiret) M. Kerguélen), Texas wintergrass (*Nassella leucotricha* (K. von Trinius and F. Ruprecht) R. Pohl), and meadow dropseed (*Sporobolus compositus* (J. Poiret) E. Merrill) (unpublished data, Welder Wildlife Foundation).

Sand and sandy loam soils served as the basis for the live oak habitat (Drawe et al. 1978). Live oak (*Quercus virginiana* P. Miller) was the predominant woody species. Huisache, blackbrush acacia, agarito, woolybucket bumelia, hog plum (*Colubrina*

*texensis* (J. Torrey and A. Gray) A. Gray var. *texensis*), Texas kidneywood (*Eysenhardtia texana* G. Scheele), honey mesquite, and lime pricklyash were also important woody plants. Important forbs included spiny aster, huisache daisy (*Amblyolepis setigera* A. P. de Candolle), plains coreopsis (*Coreopsis tinctoria* T. Nuttall var. *tinctoria*), slender croptilon (*Croptilon divaricatum* (T. Nuttall) C. Rafinesque-Schmaltz), wooly croton (*Croton capitatus* A. Michaux), Texas croton (*Croton texensis* (J. Klotzch) J. Müller of Aargau var. *texensis*), wild gourd (*Cucurbita texana* A. Gray), and silverleaf sunflower (*Helianthus argophyllus* J. Torrey and A. Gray). Rescuegrass (*Bromus catharticus* M. A. Vahl), Pan American balsamscale (*Elionurus tripsacoides* F. von Humboldt and A. Bonpland *ex* C. von Willdenow var. *tripsacoides*), brownseed paspalum (*Paspalum plicatulum* A. Michaux var. *plicatulum*), seacoast bluestem (*Schizachyrium scoparium*(A. Michaux) G. Nash var. *littorale* (G. Nash) F. Gould), and knotroot bristlegrass were the main grasses (unpublished data, Welder Wildlife Foundation).

The grassland community occurred on the same soils and consisted of the same forbs and grasses as described for the live oak community (Drawe et al. 1978). However, very few woody plants were present.

Soils in the woodland habitat were complex, changing over short distances (Drawe et al. 1978). Woody species, including huisache, netleaf hackberry (*Celtis laevigata* C. von Willdenow var. *reticulata* (J. Torrey) L. Benson), hog plum, anaqua (*Ehretia anacua* (M. Terán and J. Berlandier) I. M. Johnston), Texas kidneywood, and western soapberry (*Sapindus saponaria* C. Linnaeus var. *drummondii* (W. Hooker and

G. Arnott) L. Benson), formed a dense canopy, with mustang grape (*Vitis mustangensis* S. Buckley) draped over many of the trees. Spiny aster was the dominant forb in some areas, while dominant grasses included broadleaf chasmanthium (*Chasmanthium latifolium* (A. Michaux) H. Yates), Virginia wildrye (*Elymus virginicus* C. Linnaeus var. *virginicus*), southwestern bristlegrass (*Setaria scheelei* (E. von Steudel) A. Hitchcock), and Texas wintergrass (unpublished data, Welder Wildlife Foundation).

Plants of aquatic sites included clubhead cutgrass (*Leersia hexandra* O. Swartz), creeping lovegrass (*Eragrostis reptans* (A. Michaux) C. Nees von Esenbeck), longtom (*Paspalum lividum* K. von Trinius), bulrush (*Schoenoplectus americanus* (C. Persoon) A. von Volkart *ex* H. Schinz and R. Keller), and knotroot bristlegrass (unpublished data, Welder Wildlife Foundation). Typically dense stands of spiny aster developed around the edges with open aquatic vegetation near the center.

Disturbed areas occurred along roads and fireguards that were frequently mowed or plowed. Plants included huisache, false willow (*Baccharis neglecta* N. Britton), King ranch bluestem (*Bothriochloa ischaemum* (C. Linnaeus) Y. Keng var. *ischaemum*), common bermudagrass (*Cynodon dactylon* (C. Linnaeus) C. Persoon var. *dactylon*), false ragweed (*Parthenium hysterophorus* C. Linnaeus), and knotroot bristlegrass (unpublished data, Welder Wildlife Foundation).

Once the classification was complete, the grid coverage was modified to meet minimum patch size requirements. Four coverages were created using minimum patch sizes of 625 $m^2$ (1 grid cell), 1250 $m^2$ (2 grid cells), 2500 $m^2$ (4 grid cells), and 5000 $m^2$ (8 grid cells). Patches smaller than the minimum patch size were dissolved into

surrounding larger patches or combined to form a larger patch using several rules. First, adjacent cells of the same patch type were combined (Figure 1). Second, if a patch did not meet the minimum size requirements and was entirely surrounded by cells of one patch type, then that cell was dissolved into the surrounding patch type (became that patch type). The remaining rules were based on the assumption that similar patch types were combined when possible (see similarity groupings, Table 1). Third, the fewest number of changes were made. Fourth, the patch in question was changed to the patch type with the largest number of fully adjacent cells (Figure 1), or if the number of fully adjacent cells were equal, to the patch type with the most corner cells. If the number of corner cells was also equal, the patch was combined with the most similar adjacent habitat type (see similarity groupings, Table 1). For example, brushland-grassland would become open brushland, grassland, or woodland, open brushland would become dense brushland or brushland-grassland, etc. (Table 1).

I quantified the landscape patterns delineated in the classifications at the class scale using FRAGSTATS (McGarigal and Marks 1995), a spatial pattern analysis program. Class refers to all the patches of a particular patch type. I selected the eight-cell neighbor rule, so patch membership was based on all eight adjacent cells (versus just the four adjacent cells that share a full side) (McGarigal and Marks 1995). Analyzed data included the area of each habitat, the percent each habitat comprised of the landscape, the number of patches of each habitat, the mean patch size of each habitat, and the interspersion and juxtaposition index for each habitat. The interspersion and juxtaposition index was based on patch adjacencies, providing a measure of the

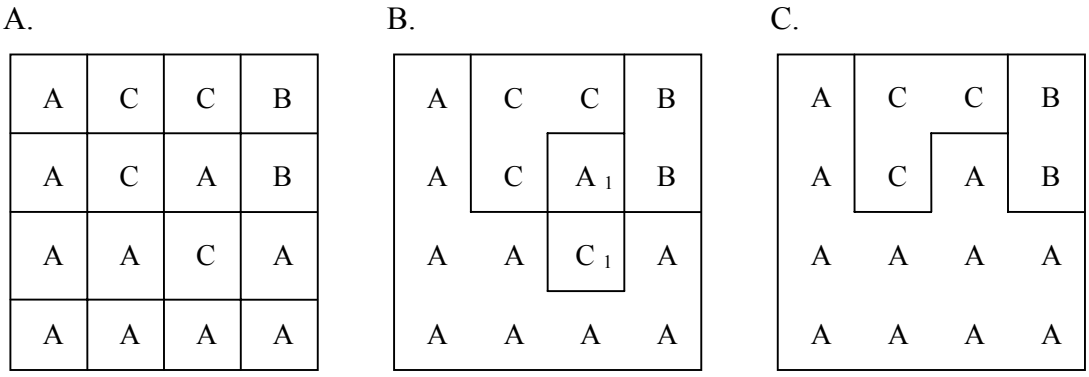



Figure 1. Modifications for minimum patch size requirements. Part A shows the original classified grid before any modifications (minimum patch size of 625 $m^2$ (1 cell)). Part B shows the application of the first rule where cells of the same patch type were combined. Part C shows the application of the fourth rule with a minimum patch size of 1250 $m^2$ (2 cells). The third rule was not applicable because an equal number of changes would have occurred by dissolving $A_1$ into C or $C_1$ into A. However, $A_1$ is fully adjacent to three cells of C (including $C_1$), while $C_1$ is fully adjacent cells to four cells of A (including $A_1$). Therefore, $C_1$ and $A_1$ become A.

interspersion or intermixing of patch types (McGarigal and Marks 1995). The classifications were converted from shape files to grid files using the Spatial Analyst extension in ArcView® GIS 3.2 and imported into FRAGSTATS. A five meter grid size was selected for each classification during the conversion process.

Class area is the sum of the areas of all patches of a particular patch type. The percentage of landscape is the proportion of the landscape of a particular class. The number of patches is the number of patches of a particular class. The mean patch area is the mean area of all patches in a class. The interspersion and juxtaposition index (IJI) is the observed interspersion divided by the maximum possible interspersion based on the number of patch types as defined by

$$IJI = \frac{-\sum_{k=1}^{m}\left[\left(\frac{e_{ik}}{\sum_{k=1}^{m} e_{ik}}\right)\ln\left(\frac{e_{ik}}{\sum_{k=1}^{m} e_{ik}}\right)\right]}{\ln(m-1)}(100),$$

where $e_{ik}$ is the total length of edge between patch types i and k and m is the number of patch types in the landscape (McGarigal and Marks 1995). IJI is close to 0 when the patch type of interest is only adjacent to one other patch type, and is equal to 100 if the patch type is equally adjacent to all other patch types. In other words, IJI increases as interspersion and juxtaposition increase.

Nectar classification

Based on the vegetation community classification described above, I developed a classification of monthly nectar availability by habitat type for the Welder Wildlife Refuge. The classification was based on the importance of different plants as nectar sources, estimates of nectar production, plant abundance, and plant growth form. Nectar importance was based on information obtained from Pellett (1977) and Sanborn and Scholl (1908) about the value of different plants as honey sources. Each plant on the Welder plant list[1] (unpublished data, Welder Wildlife Foundation) was ranked as excellent (referred to in Pellett (1977) as a main source for Texas), good (referred to in Pellett (1977) as an important source), fair (referred to in Pellett (1977) as an unimportant source or no information on importance was given), or not a source. Nectar importance by plant was then converted into conservative estimates of nectar production in terms of mg of sugar, which combines nectar volume and concentration into a single value. The range of sugar values reported in Simpson (1977) were used to place poor, fair, good, and excellent nectar sources in general categories of nectar production (Table 2), assuming more valuable nectar plants produced more nectar. The mg of sugar available per flower or inflorescence was multiplied by conservative estimates of flower production by abundance values and growth form (one flower for a herbaceous plant or ten flowers for a woody plant) (Table 2). Abundance data from the Welder plant list (unpublished data, Welder Wildlife Foundation) classified each plant as abundant (> 5 %

[1] The Welder plant list contains commonly encountered plants on the Welder Wildlife Refuge based on cover data from point frame transects surveyed from 1975 through 1984 by D. Lynn Drawe.

Table 2. Values assigned for nectar importance, pollen importance, plant abundance, and plant growth form.

| importance | mg sugar or pollen* | abundance | value** | growth form | value |
|---|---|---|---|---|---|
| excellent | 10 | abundant | 100 | forbs | 1 |
| good | 2 | frequent | 50 | grasses | 1 |
| fair | 1 | occasional | 10 | cacti | 1 |
| poor | 0.5 | rare | 0 | woody plants | 10 |

* amount per flower or inflorescence

** per grid cell (625 $m^2$)

cover), frequent (1-5 % cover), occasional (0-1 % cover), or rare (not encountered during sampling, but seen along the sampling transect). Therefore, an occasional forb would have 10 flowers per cell (625 $m^2$), an occasional woody plant would have 100 flowers per cell, a frequent forb would have 50 flowers per cell, etc. Individual cell values for each habitat type were multiplied by the number of cells of that habitat type within the study area to provide overall estimates for each habitat type. Flowering periods reported in Jones (1982) were used to establish when and for how long plants secreted nectar. These estimates are very conservative and probably represent the minimum nectar potential for the study site. For example, a flowering honey mesquite may produce thousands of inflorescences per day when in bloom (personal observation). However, an individual plant typically does not bloom for the entire length of the reported flowering period, and the number of flowers produced per day is variable. Therefore, these estimates of the mg of sugar produced are intended to average out over the blooming period of a plant to provide a very conservative estimate of the total nectar produced, which can be used to identify if and when resources may be limiting for feral honey bees on the Welder Wildlife Refuge.

In some cases, the habitats identified in the landscape classification were combined in the Welder plant list (unpublished data, Welder Wildlife Foundation) because they occurred on the same soil types. Dense brushland, open brushland, and brushland-grassland were grouped together on clay and clay loam soils, and grassland, open live oak, and dense live oak were grouped together on sand and sandy loam soils. In order to separate these habitats, I weighted the values for grasses and forbs and the

values for woody species. The grasses and forbs in the grassland habitats (brushland-grassland and grassland) were increased by 50 % and the woody species decreased by 50 %. The grasses and forbs in the dense habitats (dense brushland and dense live oak) were decreased by 50 % and the woody species increased by 50 %. These changes were chosen based on knowledge about the plant communities on the Welder Wildlife Refuge (D. Lynn Drawe, personal communication). The open habitats (open brushland and open live oak) were considered the default habitats with no weights applied (weighting values equal to one).

Nectar availability was calculated for each month and habitat type based on the blooming periods of the different plants (Jones 1982). Once the rankings were developed, the values were divided into four groups and designated as poor, fair, good, or excellent for nectar availability. These groupings were based on the maximum value, with equal intervals ranging from 0 to 25 %, > 25 % to 50 %, > 50 % to 75 %, and > 75 % to 100 % of the maximum. Therefore, these rankings compared nectar availability between months and habitat types on an annual basis. Nectar availability was also compared between habitat types within a month by reassigning the group membership based on the maximum value for each month.

I also estimated the amount of sugar available within the cumulative foraging range of all the colonies within the study area, since the foraging ranges of most of the colonies extended beyond the study area. The coordinates for each cavity tree used by a feral colony during the past twelve years were recorded to a submeter accuracy using a Trimble GPS Pathfinder™ receiver and TSC1™ Asset Surveyor™ data logger. Based

on these coordinates, the foraging area of each cavity was estimated as a circle with a radius of 800 m, since mean foraging distances range from approximately 500 m to over 2000 m, depending on the foraging environment and colony conditions (Gary et al. 1972, Visscher and Seeley 1982, Schneider 1989, Schneider and McNally 1993, Waddington et al. 1994, Schneider and Hall 1997, Beekman and Ratnieks 2000). The foraging ranges around each cavity overlapped with those of surrounding cavities, so they were combined into a single polygon to estimate a cumulative foraging area for the population of feral colonies on the Welder Wildlife Refuge. Then, the area of this polygon (the cumulative foraging range) that extended beyond the study site boundaries was measured. Assuming the area outside the study site was similar to the study site, the percentage the outside area comprised of the study site was multiplied by the mg of sugar produced in the study site and added to the total value for the study site to provide an estimate for the expanded foraging area of the colonies.

The number of colonies that could be supported by the mg of sugar produced within the study site and the expanded foraging area was estimated on an annual basis. The amount of honey required by a colony was assumed to be 97 kg per year, as estimated by Buchmann et al. (1992) for feral colonies.

## Pollen classification

Based on the vegetation community classification described above, I developed a classification of monthly pollen availability by habitat type for the Welder Wildlife Refuge. Pollen importance was based on triweekly pollen collection data from feral

colonies on the Welder Wildlife Refuge (unpublished data, K. A. Baum[2], W. L. Rubink, and R. N. Coulson,) and information obtained from Pellett (1977) about the value of different plants as pollen sources. Each plant on the Welder plant list (unpublished data, Welder Wildlife Foundation) was ranked as excellent (predominant pollen source at Welder), good (secondary pollen source at Welder), fair (important minor pollen source at Welder), or poor (minor pollen source at Welder). Predominant pollen sources comprised > 45 % of a sample averaged across all colonies for a single sampling period, secondary pollen sources comprised 16 – 45 % of a sample, important minor pollen sources comprised 3 – 15 % of a sample, and minor pollen sources made up < 3 % of a sample (Louveaux et al. 1978). Pollen sources listed in Pellett (1977) and on the Welder plant list (unpublished data, Welder Wildlife Foundation), but not collected by the feral colonies during the sampling periods, were added. Any pollen sources listed as more important in Pellett (1977) compared to the Welder pollen collection data were increased in value, since the three-week sampling regime may not have captured sources the colonies used for only brief periods of time. Pollen importance was then converted into conservative estimates of pollen production in terms of mg of pollen. The range of pollen values reported in Simpson (1977) were used to place poor, fair, good, and excellent pollen sources in general categories of pollen production. The mg of pollen available per flower or inflorescence was multiplied by abundance values and growth form (Table 2). The overall rationale and methodology for these calculations follows

[2] Refers to data provided in other chapters of this dissertation.

that described for the equivalent nectar quantity (mg of sugar) estimates and the expanded foraging area calculations and corresponding estimates. Weighting factors were also the same as described for the nectar classification. The final estimates of pollen availability for each month and habitat type were plotted and four groups were identified as described for the nectar classification, and designated as poor, fair, good, or excellent for pollen availability. These rankings compared pollen availability between months and habitat types on an annual basis.

Pollen availability was calculated for each month and habitat type based on the blooming periods of different plants (Jones 1982). These values were plotted and four groups were identified as described for the nectar classification, and assigned overall rankings of poor, fair, good, or excellent for pollen availability. These rankings compared pollen availability between habitat types within a month.

The number of colonies that could be supported by the mg of pollen produced within the study site and the expanded foraging area was estimated on an annual basis. The amount of pollen required by a colony was based on a mean of 26.5 kg of pollen collected per year by managed colonies by Buchmann et al. (1992) (at a 60 % trap efficiency) and the assumption that feral colonies would consume about 50 % as much pollen as larger managed colonies (Buchmann et al. 1992).

Cavity classification

I developed a classification of cavity availability by habitat type for the Welder Wildlife Refuge. For the purposes of this study, cavity availability refers only to cavities

suitable for feral honey bee colonies. Because cavity availability would not vary seasonally, only one classification was developed that applied to all months. The importance of a tree species as a cavity source was based on data about feral colony use of cavities collected on the Welder Wildlife Refuge (unpublished data, W. L. Rubink). Feral colonies were found to use cavities in five types of trees, and these species were ranked according to cavity use. Therefore, *Quercus virginiana* was considered an excellent cavity source, *Celtis* spp. a good source, *Ehretia anacua* a fair source, and *Ulmus crassifolia* T. Nuttall and *Morus rubra* C. Linnaeus poor sources. Overall rankings of poor, fair, good, or excellent were assigned to each habitat type for cavity availability as described for the nectar and pollen classifications. These rankings compared cavity availability between habitat types on an annual basis.

Combined classification

NetWeaver™ was used to define a rule base about feral honey bee behavior in coastal prairie landscapes. NetWeaver™ is an object oriented software application that facilitates the organization of a knowledge base using dependency networks (Miller and Saunders 2002). A knowledge base is a set of rules that defines the relationships among components identified as part of the system of interest. Dependency networks define logical relationships between these components that explain how the system of interest functions. NetWeaver™ also identifies inconsistencies within the dependency networks and allows the user to implement fuzzy logic for defining relationships where abrupt transitions between true or false arguments are not realistic (Miller and Saunders 2002).

The rule base for feral honey bee behavior was defined in relation to the nectar, pollen, and cavity classifications. Water sources were not included because water is generally abundant in the study area and some sources (cattle tanks) were not associated with particular habitat types and were not large enough to meet the classification criteria. The northern boundary of the refuge is the Aransas River and there are several natural lakes and wetlands, as well as human made tanks throughout the study area. Overall nectar and pollen classifications were developed that combined nectar and pollen availability throughout the year for one overall ranking. Excellent, good, fair, and poor rankings received values of four, three, two, and one, respectively.

The rule base assumed that higher quality resources provided more suitable habitat for feral honey bees. To avoid constraints created by cavity locations (cavities were the only resource absent from some habitats), the average of the combined rankings for pollen, nectar, and cavities was used (Figure 2). Avoiding cavity constraints seems reasonable for evaluating the suitability of habitat for feral honey bees at the scale of the study area, since workers forage anywhere from a few meters to over 10000 meters from the colony and the study area is only about 2500 m by 2500 m (Gary et al. 1972, Visscher and Seeley 1982, Schneider 1989, Schneider and McNally 1993, Waddington et al. 1994, Schneider and Hall 1997, Beekman and Ratnieks 2000). The same rationale also would apply to nectar and pollen if they should be limiting in a habitat. Therefore, habitats with different combinations of nectar, pollen, and cavity availability were considered excellent, good, fair, poor, or not habitat for feral colonies based on the average of their cumulative rankings (Figure 2). I used GeoNetWeaver$^{TM}$, a version of

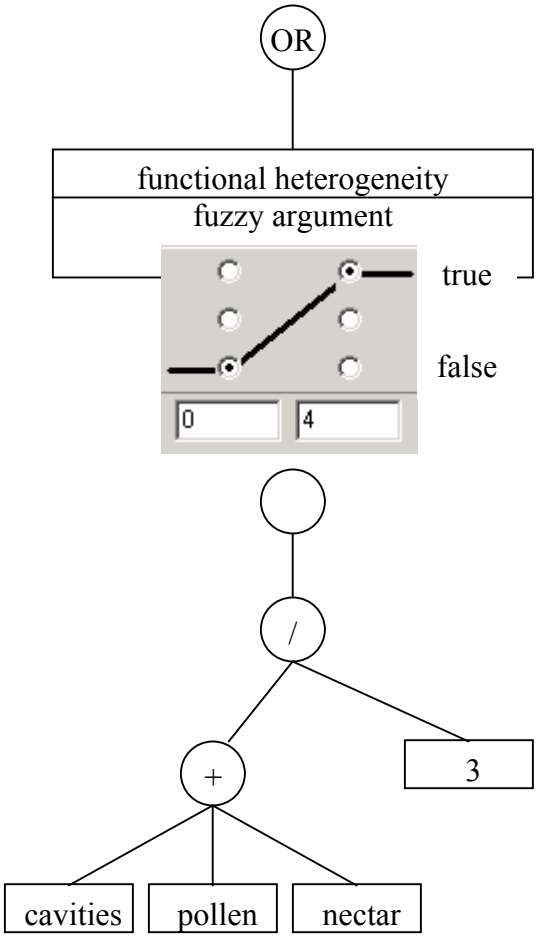



Figure 2. The NetWeaver™ dependency network for the knowledge base on feral honey bee behavior in coastal prairie landscapes. Values for cavity, pollen, and nectar sources ranged from 0 (not habitat) to 4 (excellent habitat). The average value for cavity, pollen, and nectar sources in a habitat were evaluated using a fuzzy argument with values ranging from 0 (false) to 4 (true).

NetWeaver™ applied to spatially referenced data, to implement the rule base. I developed maps showing the overall quality of different areas of the Welder Wildlife Refuge for feral honey bees in ArcView® GIS 3.2.

## RESULTS

### Landscape classification and quantification

Grassland was the most abundant habitat type, followed by open live oak, woodland, brushland-grassland, and open brushland (Table 3, Figure 3). The least common habitat types were disturbed, aquatic plants, and water. The habitats with the most patches were grassland, dense live oak, and brushland-grassland. Woodland contained the largest patches, followed by open live oak. The water, aquatic plants, and disturbed habitats contained the smallest patches. Woodland and grassland were the most interspersed and juxtaposed, while dense live oak showed the least interspersion and juxtaposition (Table 3, Figure 3).

### Nectar classification

Important nectar plants varied by month and habitat (Table 4). However, *Prosopis glandulosa* was the most important nectar source, flowering for much of the year in the seven largest habitat types. Nectar availability was poor from October through February in all habitat types (Table 5). In March through September, dense live oak had the highest nectar availability, followed by open live oak, open brushland, and

Table 3. Selected class metrics for the 5000 $m^2$ minimum patch size classification. Class area is the area of each habitat, % of landscape is the percent each habitat comprised of the landscape, # of patches is the number of patches of each habitat, mean patch area is the mean patch area of each habitat, and the interspersion and juxtaposition index provides a measure of the interspersion or intermixing of patch types.

| classes | class area (ha) | % of landscape | # of patches | mean patch area (ha) | interspersion/juxtaposition (%) |
|---|---|---|---|---|---|
| aquatic plants | 5.00 | 0.86 | 3 | 1.67 | 58.48 |
| brushland (dense) | 28.19 | 4.87 | 12 | 2.35 | 60.88 |
| brushland (open) | 80.75 | 13.94 | 12 | 6.73 | 59.36 |
| brushland-grassland | 82.56 | 14.25 | 15 | 5.50 | 46.98 |
| disturbed | 4.13 | 0.71 | 4 | 1.03 | 63.89 |
| grassland | 112.50 | 19.42 | 18 | 6.25 | 81.89 |
| live oak (dense) | 65.44 | 11.30 | 17 | 3.85 | 37.08 |
| live oak (open) | 107.81 | 18.61 | 10 | 10.78 | 60.27 |
| water | 8.63 | 1.49 | 9 | 0.96 | 51.99 |
| woodland | 84.25 | 14.54 | 5 | 16.85 | 85.39 |

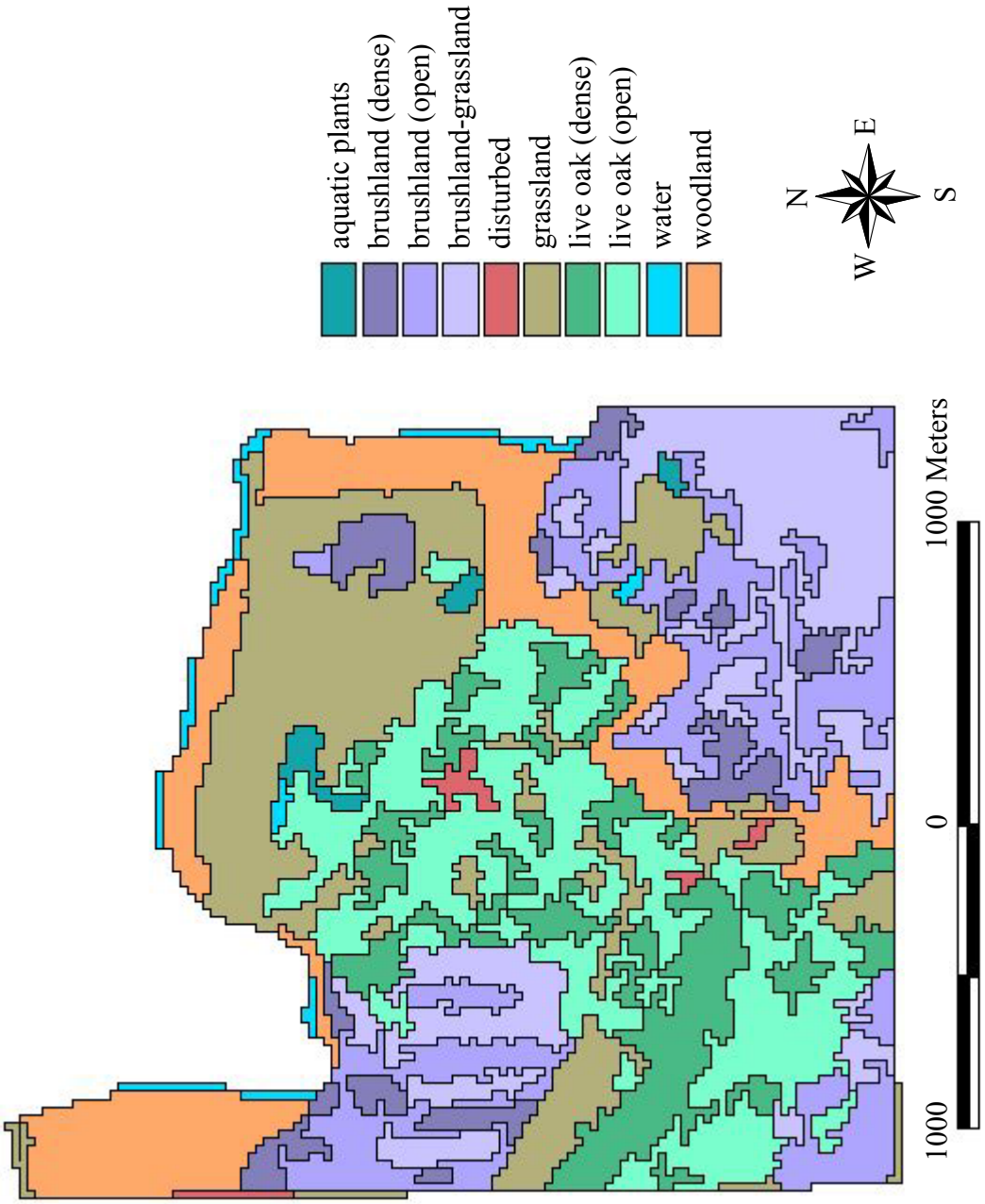



Figure 3. Vegetation community classification at the 5000 m2 minimum patch size.

Table 4. Important nectar sources by habitat type and month.

| habitat type | genus and species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aquatic plants | *Lycium carolinianum* | | | | | | | | | | | | |
| | *Nelumbo* (*N. lutea*) | | | | | | | | | | | | |
| | *Sagittaria* spp. | | | | | | | | | | | | |
| brushland group (dense, open, and brusland-grassland) | *Acacia rigidula* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Celtis pallida* | | | | | | | | | | | | |
| | *Diospyros texana* | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| | *Zanthoxylum fagara* | | | | | | | | | | | | |
| disturbed | *Croton monanthogynus* | | | | | | | | | | | | |
| | *Opuntia leptocaulis* | | | | | | | | | | | | |
| | *Senicio imparipinnatus* | | | | | | | | | | | | |
| | *Sorghum halepense* | | | | | | | | | | | | |
| live oak (dense and open) | *Acacia rigidula* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Bumelia lanuginosa* | | | | | | | | | | | | |
| | *Colubrina texensis* | | | | | | | | | | | | |

Table 4. Continued.

| habitat type | genus and species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| live oak (dense and open) | *Eysenhardtia texana* | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| | *Prunus* (*P. texana*) | | | | | | | | | | | | |
| | *Quercus virginiana* | | | | | | | | | | | | |
| | *Zanthoxylum fagara* | | | | | | | | | | | | |
| grassland | *Acacia rigidula* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Bumelia lanuginosa* | | | | | | | | | | | | |
| | *Colubrina texensis* | | | | | | | | | | | | |
| | *Cucurbita texana* | | | | | | | | | | | | |
| | *Eysenhardtia texana* | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| | *Prunus* (*P. texana*) | | | | | | | | | | | | |
| | *Quercus virginiana* | | | | | | | | | | | | |
| | *Zanthoxylum fagara* | | | | | | | | | | | | |
| woodland | *Celtis reticulata* | | | | | | | | | | | | |
| | *Colubrina texensis* | | | | | | | | | | | | |

Table 4. Continued.

| habitat type | genus and species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| woodland | *Ehretia anacua* | | | | | | | | | | | | |
| | *Eysenhardtia texana* | | | | | | | | | | | | |
| | *Fraxinus* spp. | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| | *Rhus* (*R. toxicodendron*) | | | | | | | | | | | | |
| | *Sapindus saponaria* | | | | | | | | | | | | |
| | *Vitis mustangensis* | | | | | | | | | | | | |

Table 5. Nectar availability between months and habitat types. Shading emphasizes the availability rankings from lightest (poor) to darkest (excellent).

| month | aquatic plants | brushland (dense) | brushland (open) | brushland-grassland | disturbed | grassland | live oak (dense) | live oak (open) | woodland |
|---|---|---|---|---|---|---|---|---|---|
| Jan | poor | poor | poor | poor | poor | poor | poor | poor | poor |
| Feb | poor | poor | poor | poor | poor | poor | poor | poor | poor |
| Mar | poor | fair | good | fair | poor | good | excellent | excellent | fair |
| Apr | poor | fair | good | fair | poor | good | excellent | excellent | fair |
| May | poor | fair | good | fair | poor | good | excellent | excellent | fair |
| Jun | poor | fair | good | fair | poor | good | excellent | good | poor |
| Jul | poor | fair | good | fair | poor | good | excellent | good | poor |
| Aug | poor | fair | good | fair | poor | fair | excellent | good | poor |
| Sep | poor | fair | good | fair | poor | good | excellent | good | poor |
| Oct | poor | poor | poor | poor | poor | poor | poor | poor | poor |
| Nov | poor | poor | poor | poor | poor | poor | poor | poor | poor |
| Dec | poor | poor | poor | poor | poor | poor | poor | poor | poor |

grassland. Dense brushland and brushland-grassland were considered fair nectar sources (Table 5).

When comparing nectar availability within months, dense brushland and dense live oak were excellent in January, and March through September (Table 6, Figures 4, 6). Dense brushland was the only excellent source of nectar in February (Table 6, Figure 5), while the grassland and open live oak were excellent sources from October through December (Table 6, Figures 7-9). Dense live oak and woodland were also excellent sources in October and November (Table 6, Figures 7-8). Open brushland and open live oak tended to be good sources of nectar throughout the year. The aquatic and disturbed habitats were never important sources of nectar (Table 6, Figures 4-9).

Based on the mg of sugar estimates (reported as kg of sugar), January and December produced the least nectar (Table 7). February, October, and November produced a moderate amount of nectar, while a large amount of nectar was produced from March through September. Nectar production was highest in March, April, and May.

Pollen classification

Important pollen plants varied by month and habitat (Table 8). However, *Prosopis glandulosa* was the most important pollen source, flowering for much of the year in the seven largest habitat types. Pollen availability was low from October through February (Table 9). Dense live oak was an excellent pollen source from March through September, while open live oak was an excellent pollen source from March through June

Table 6. Nectar availability between habitat types for each month (rankings cannot be compared between months – see Table 5 for comparisons between months). Shading emphasizes the availability rankings from lightest (poor) to darkest (excellent).

| month | aquatic plants | brushland (dense) | brushland (open) | brushland-grassland | disturbed | grassland | live oak (dense) | live oak (open) | woodland |
|---|---|---|---|---|---|---|---|---|---|
| Jan | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Feb | poor | excellent | good | fair | poor | poor | fair | fair | poor |
| Mar | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Apr | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| May | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Jun | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Jul | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Aug | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Sep | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Oct | poor | good | good | fair | poor | excellent | excellent | excellent | excellent |
| Nov | poor | good | fair | fair | poor | excellent | excellent | excellent | excellent |
| Dec | poor | fair | fair | good | poor | excellent | good | excellent | poor |

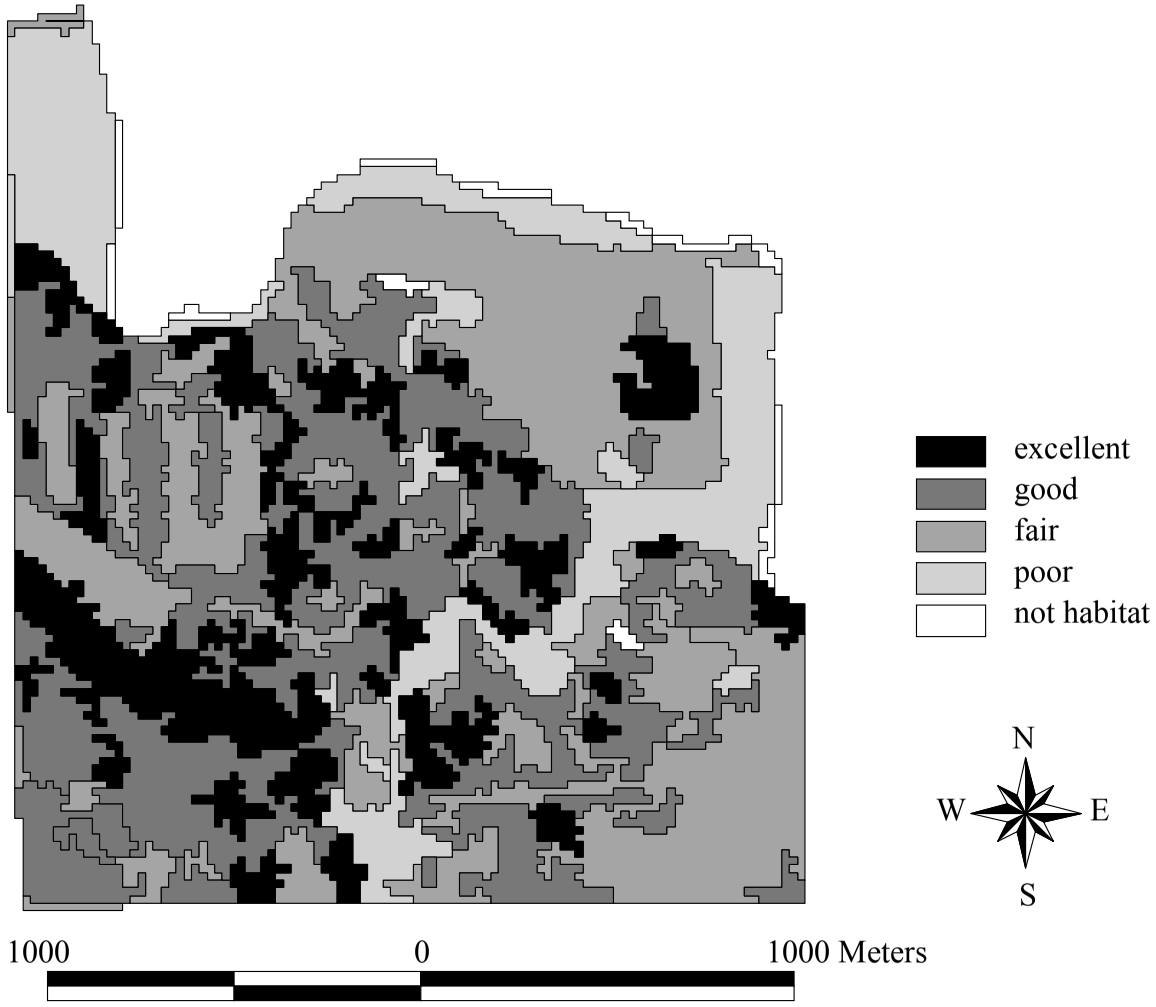



Figure 4. Nectar classification for January.

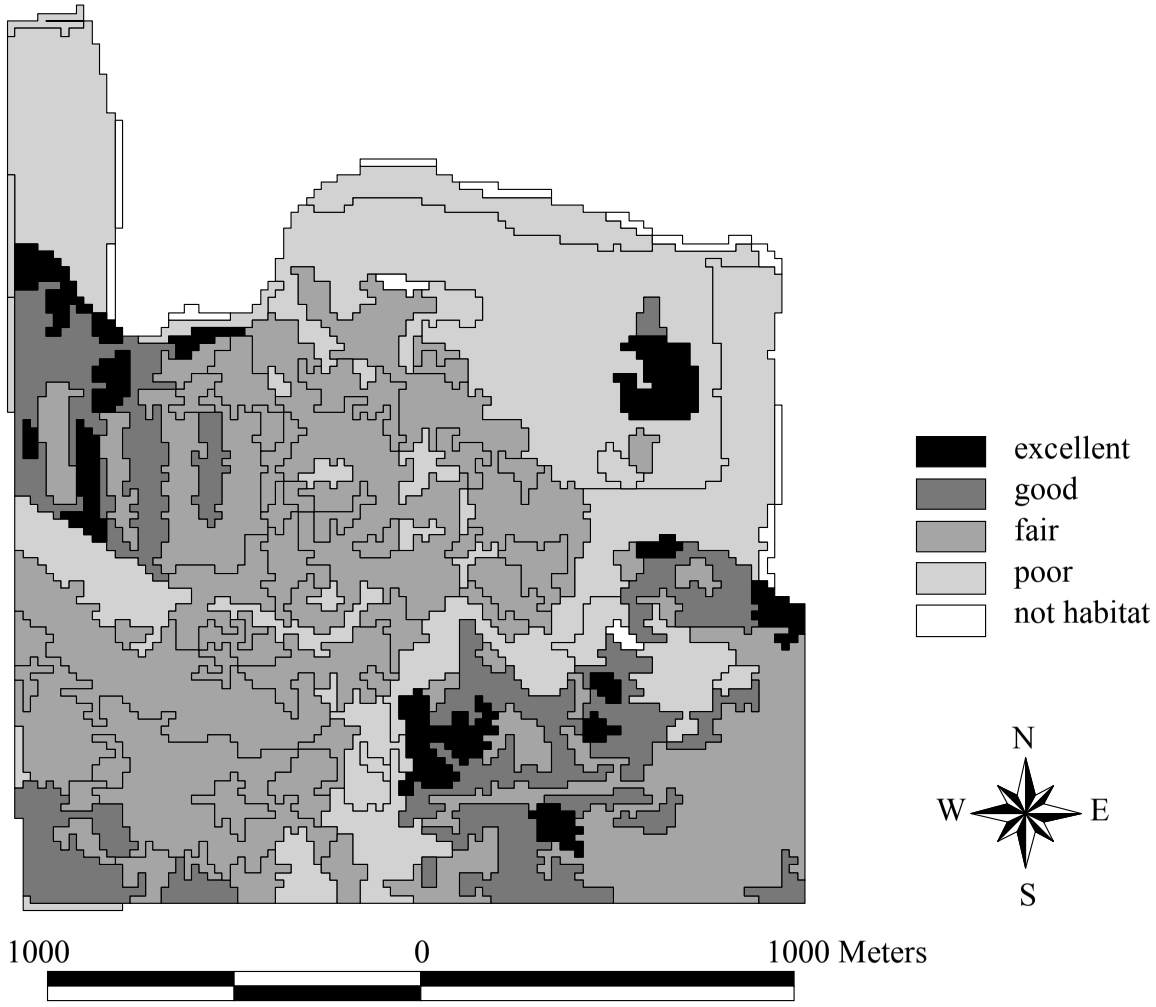



Figure 5. Nectar classification for February.

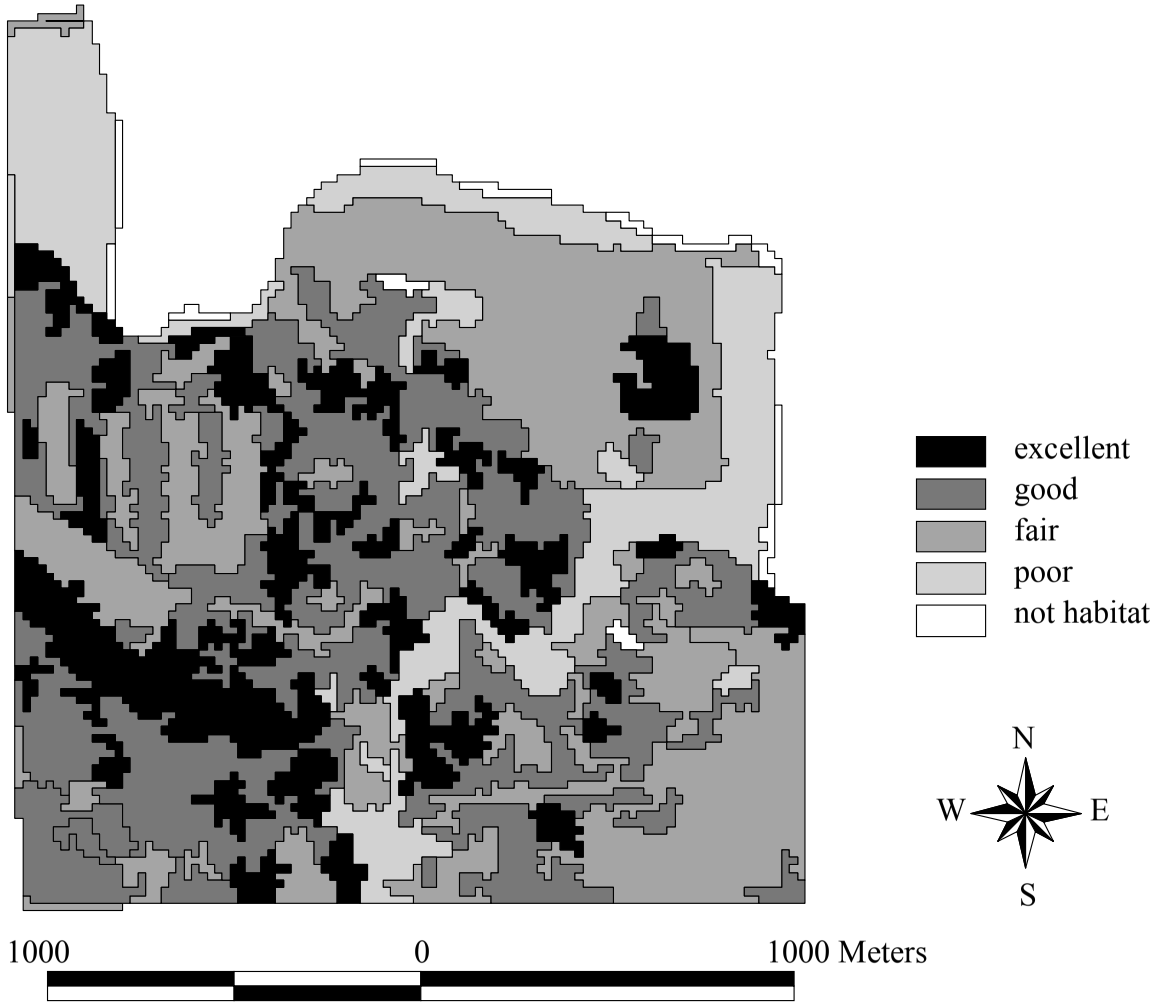



Figure 6. Nectar classification for March through September.

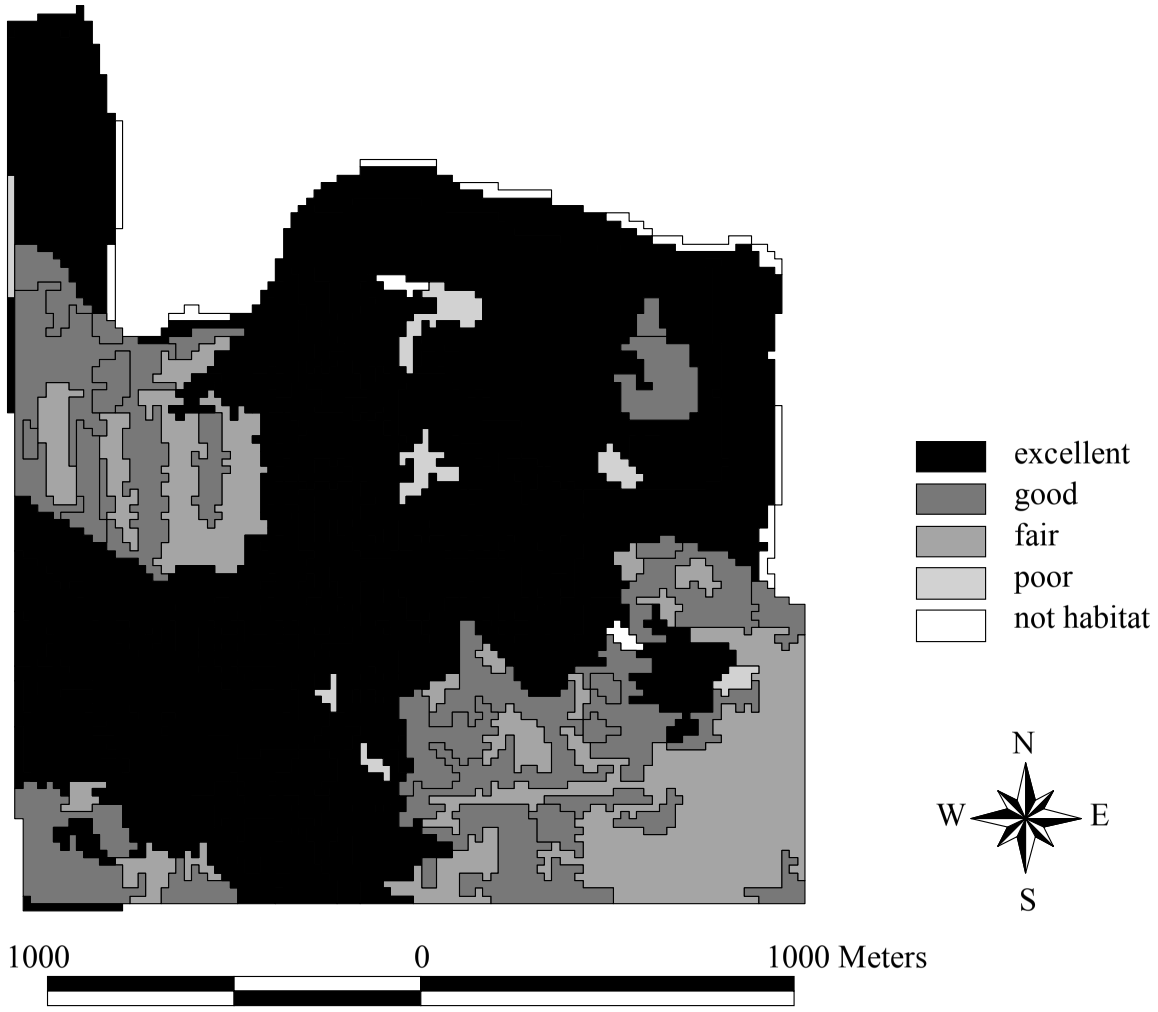



Figure 7. Nectar classification for October.

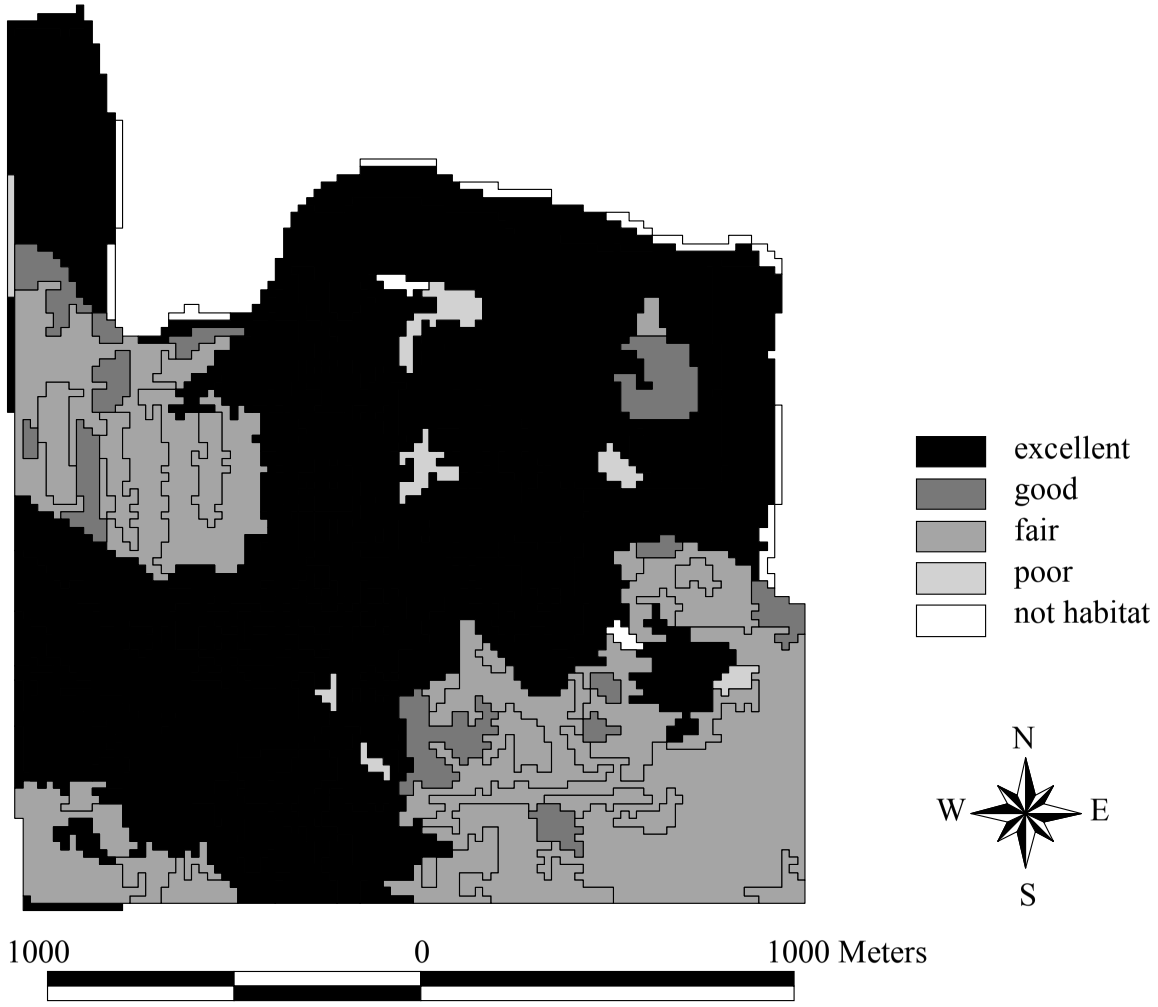



Figure 8. Nectar classification for November.

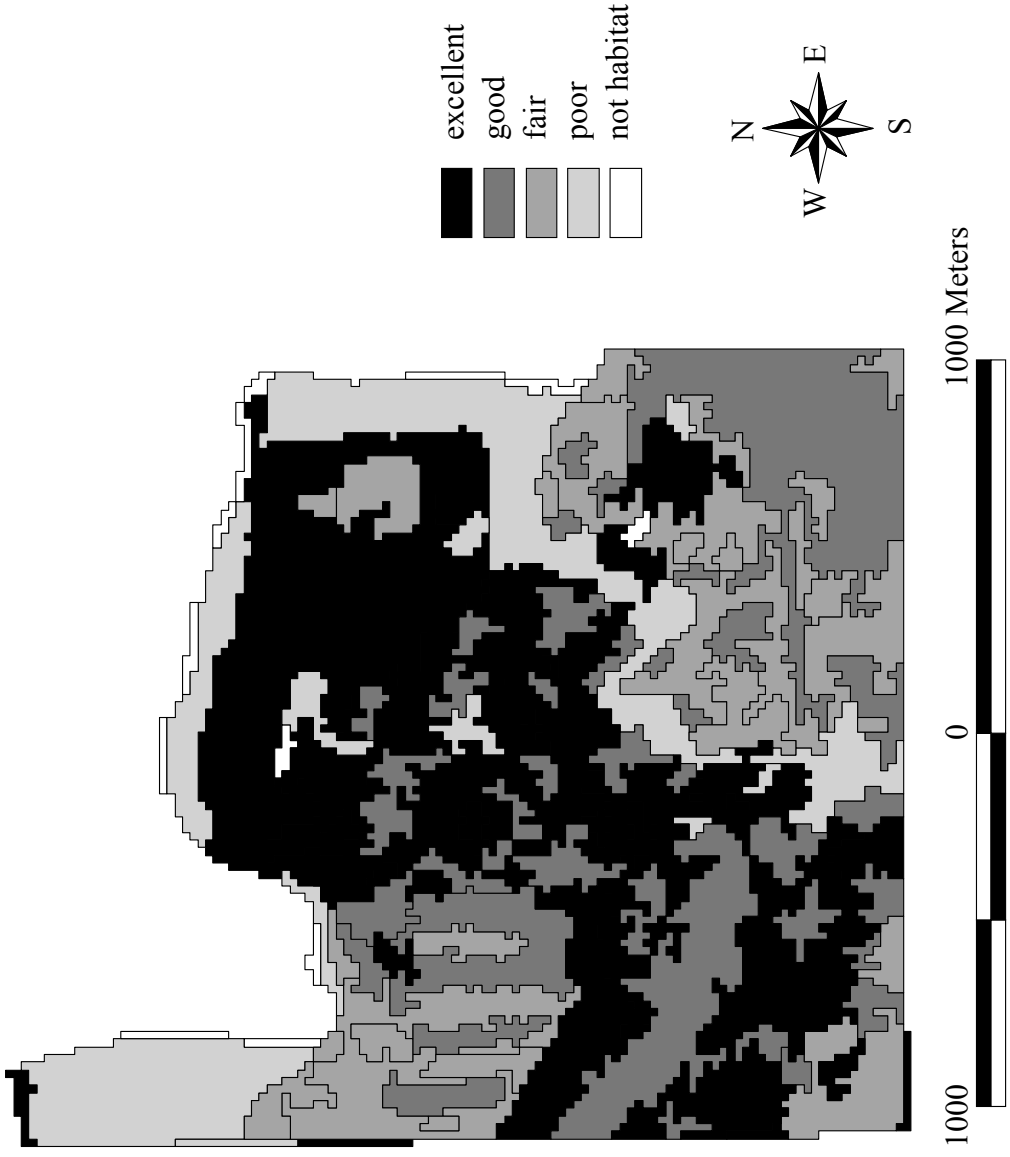



Figure 9. Nectar classification for December.

Table 7. Conservative estimates of the kg of sugar produced in the study site, as well as the kg of sugar produced within the cumulative potential foraging range of all the colonies in the study site.

| | study site only | expanded foraging area (study site + 66.79 %) |
|---|---|---|
| month | kg sugar | kg sugar |
| Jan | 127 | 211 |
| Feb | 571 | 952 |
| Mar | 3627 | 6049 |
| Apr | 3406 | 5681 |
| May | 3210 | 5355 |
| Jun | 2908 | 4851 |
| Jul | 2804 | 4676 |
| Aug | 2778 | 4633 |
| Sep | 2825 | 4711 |
| Oct | 662 | 1104 |
| Nov | 579 | 966 |
| Dec | 191 | 319 |
| annual | 23687 | 39508 |
| colonies supported | 244 | 407 |

Table 8. Important pollen sources by habitat type and month.

| habitat type | genus and species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aquatic plants | *Acer negundo* | | | | | | | | | | | | |
| | *Lycium carolinianum* | | | | | | | | | | | | |
| | *Nelumbo* (*N. lutea*) | | | | | | | | | | | | |
| | *Pedicularis* spp. | | | | | | | | | | | | |
| brushland (dense) | *Acacia farnesiana* | | | | | | | | | | | | |
| | *Acacia rigidula* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Celtis laevigata* | | | | | | | | | | | | |
| | *Celtis pallida* | | | | | | | | | | | | |
| | *Iva annua* | | | | | | | | | | | | |
| | *Prospospis glandulosa* | | | | | | | | | | | | |
| | *Prosopis reptans* | | | | | | | | | | | | |
| brushland (open) | *Acacia farnesiana* | | | | | | | | | | | | |
| | *Acacia rigidula* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Celtis pallida* | | | | | | | | | | | | |
| | *Iva annua* | | | | | | | | | | | | |

Table 8. Continued.

| habitat type | genus and species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| brushland (open) | *Propospis glandulosa* | | | | | | | | | | | | |
| brushland-grassland | *Acacia farnesiana* | | | | | | | | | | | | |
| | *Acacia rigidula* | | | | | | | | | | | | |
| | *Ambrosia psilostachya* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Celtis pallida* | | | | | | | | | | | | |
| | *Iva annua* | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| disturbed | *Acer negundo* | | | | | | | | | | | | |
| | *Cirsium texanum* | | | | | | | | | | | | |
| | *Croptilon divaricatum* | | | | | | | | | | | | |
| | *Croton capitatus* | | | | | | | | | | | | |
| | *Croton monanthogynus* | | | | | | | | | | | | |
| | *Croton texensis* | | | | | | | | | | | | |
| | *Iva annua* | | | | | | | | | | | | |
| | *Sorghum halepense* | | | | | | | | | | | | |

Table 8. Continued.

| habitat type | genus and species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| grassland | *Acacia farnesiana* | | | | | | | | | | | | |
| | *Acacia rigidula* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Colubrina texensis* | | | | | | | | | | | | |
| | *Croton capitatus* | | | | | | | | | | | | |
| | *Croton texensis* | | | | | | | | | | | | |
| | *Cucurbita texana* | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| | *Prunus* (*P. texana*) | | | | | | | | | | | | |
| | *Quercus virginiana* | | | | | | | | | | | | |
| live oak (dense and open) | *Acacia farnesiana* | | | | | | | | | | | | |
| | *Acacia rigidula* | | | | | | | | | | | | |
| | *Acer negundo* | | | | | | | | | | | | |
| | *Berberis trifoliolata* | | | | | | | | | | | | |
| | *Colubrina texensis* | | | | | | | | | | | | |
| | *Croton capitatus* | | | | | | | | | | | | |
| | *Croton texensis* | | | | | | | | | | | | |

Table 8. Continued.

| habitat type | genus and species | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| live oak (dense and open) | *Eysenhardtia texana* | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| | *Prunus* (*P. texana*) | | | | | | | | | | | | |
| | *Quercus virginiana* | | | | | | | | | | | | |
| woodland | *Acacia farnesiana* | | | | | | | | | | | | |
| | *Celtis reticulata* | | | | | | | | | | | | |
| | *Colubrina texensis* | | | | | | | | | | | | |
| | *Croton monanthogynus* | | | | | | | | | | | | |
| | *Eupatorium odoratum* | | | | | | | | | | | | |
| | *Fraxinus* spp. | | | | | | | | | | | | |
| | *Propospis glandulosa* | | | | | | | | | | | | |
| | *Quercus virginiana* | | | | | | | | | | | | |

Table 9. Pollen availability between months and habitat types. Shading emphasizes the availability rankings from lightest (poor) to darkest (excellent).

| month | aquatic plants | brushland (dense) | brushland (open) | brushland-grassland | disturbed | grassland | live oak (dense) | live oak (open) | woodland |
|---|---|---|---|---|---|---|---|---|---|
| Jan | poor | poor | poor | poor | poor | poor | poor | poor | poor |
| Feb | poor | poor | poor | poor | poor | poor | poor | poor | poor |
| Mar | poor | fair | good | fair | poor | good | excellent | excellent | poor |
| Apr | poor | fair | good | fair | poor | good | excellent | excellent | poor |
| May | poor | fair | good | fair | poor | excellent | excellent | excellent | poor |
| Jun | poor | fair | good | fair | poor | good | excellent | excellent | poor |
| Jul | poor | fair | good | fair | poor | good | excellent | good | poor |
| Aug | poor | fair | good | fair | poor | good | excellent | good | poor |
| Sep | poor | fair | good | fair | poor | good | excellent | excellent | poor |
| Oct | poor | poor | poor | poor | poor | fair | poor | poor | poor |
| Nov | poor | poor | poor | poor | poor | fair | poor | poor | poor |
| Dec | poor | poor | poor | poor | poor | poor | poor | poor | poor |

and again in September. Grassland was an excellent source in May and a fair source in October and November when all other habitats were poor sources. Open brushland and grassland were good sources of pollen throughout much of the year, while dense brushland and brushland-grassland were fair sources (Table 9).

Within months, the dense live oak habitat was the best pollen source in January (Table 10, Figure 10), followed by dense brushland and dense live oak from February through April (Table 10, Figures 11-12). Dense brushland, dense live oak, and open live oak were excellent sources from May through September (Table 10, Figure 13). For the remainder of the year (October through December), the grassland habitat was the best source of pollen (Table 10, Figures 14-16). The aquatic habitat was consistently a poor source of pollen, while the woodland and disturbed habitats occasionally provided fair amounts of pollen (Table 10, Figures 10-16).

Based on the mg of pollen estimates (reported as kg of pollen), January and December produced the least pollen, followed by February, November, and October, respectively (Table 11). An extremely large amount of pollen was produced from March through September (Table 11).

Cavity classification

The dense live oak habitat was the best source of cavities with an excellent ranking, followed by the open live oak and woodland habitats with good rankings (Table 12, Figure 17). Dense and open brushland were considered fair sources, while brushland-grassland and grassland were poor sources (Table 12, Figure 17).

Table 10. Pollen availability between habitat types for each month (rankings cannot be compared between months – see Table 9 for comparisons between months). Shading emphasizes the availability rankings from lightest (poor) to darkest (excellent).

| month | aquatic plants | brushland (dense) | brushland (open) | brushland-grassland | disturbed | grassland | live oak (dense) | live oak (open) | woodland |
|---|---|---|---|---|---|---|---|---|---|
| Jan | poor | fair | fair | poor | poor | fair | excellent | good | fair |
| Feb | poor | excellent | good | fair | poor | fair | excellent | good | fair |
| Mar | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| Apr | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| May | poor | excellent | good | fair | poor | good | excellent | excellent | poor |
| Jun | poor | excellent | good | fair | poor | good | excellent | excellent | poor |
| Jul | poor | excellent | good | fair | poor | good | excellent | excellent | poor |
| Aug | poor | excellent | good | fair | poor | good | excellent | excellent | poor |
| Sep | poor | excellent | good | fair | poor | good | excellent | excellent | poor |
| Oct | poor | fair | good | good | fair | excellent | fair | good | fair |
| Nov | poor | fair | fair | good | fair | excellent | fair | good | fair |
| Dec | poor | poor | fair | fair | fair | excellent | fair | good | fair |

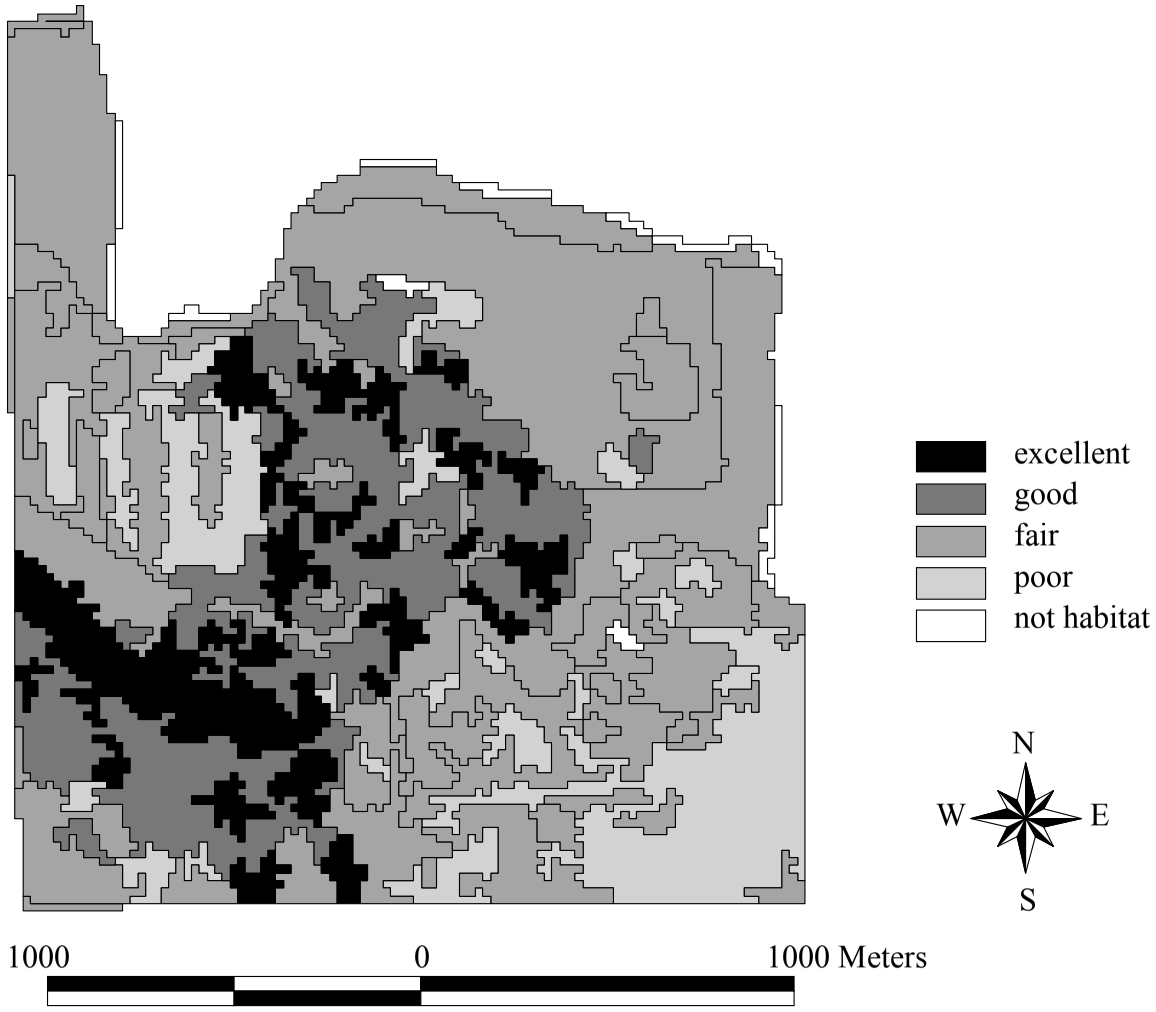



Figure 10. Pollen classification for January.

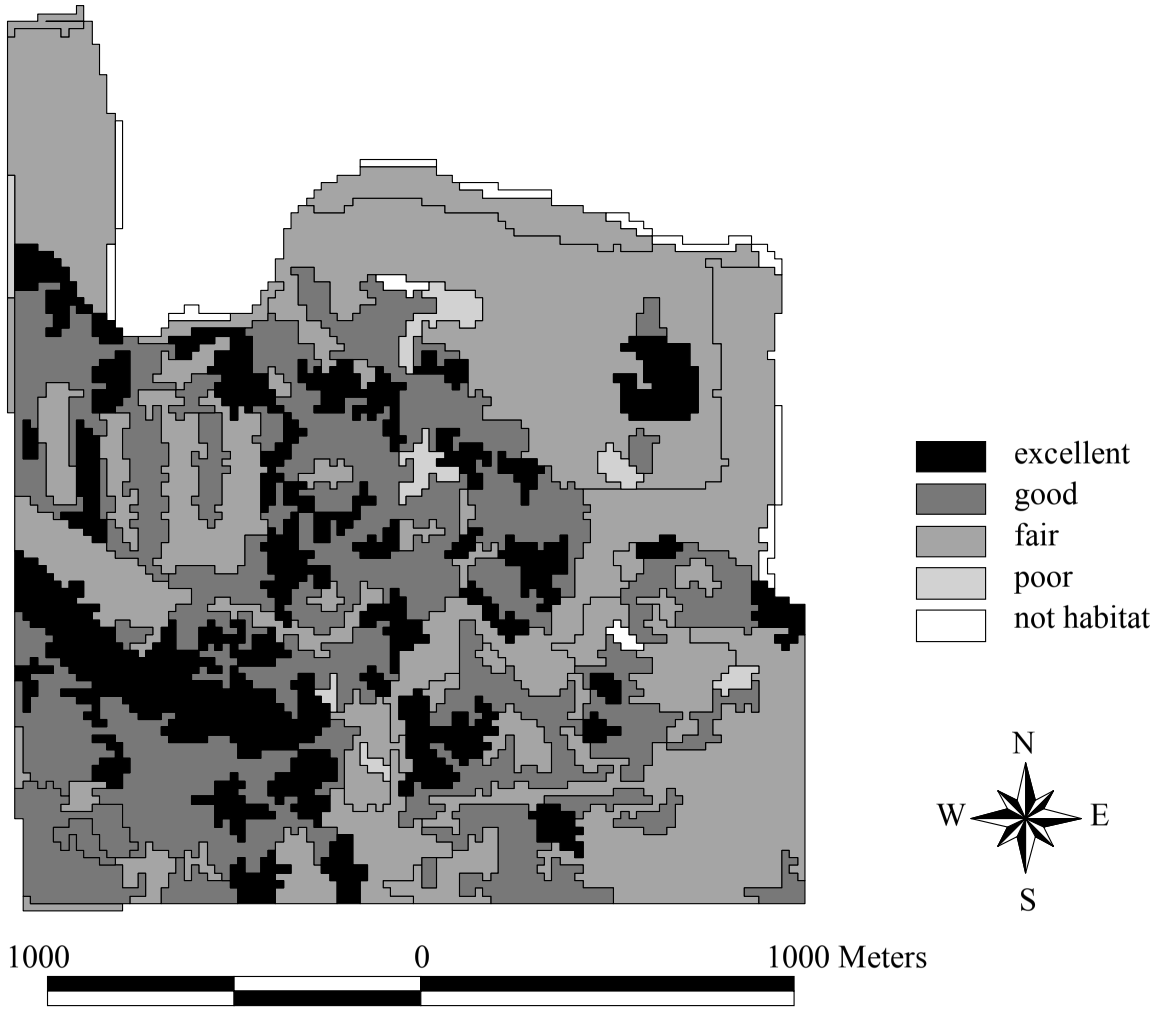



Figure 11. Pollen classification for February.

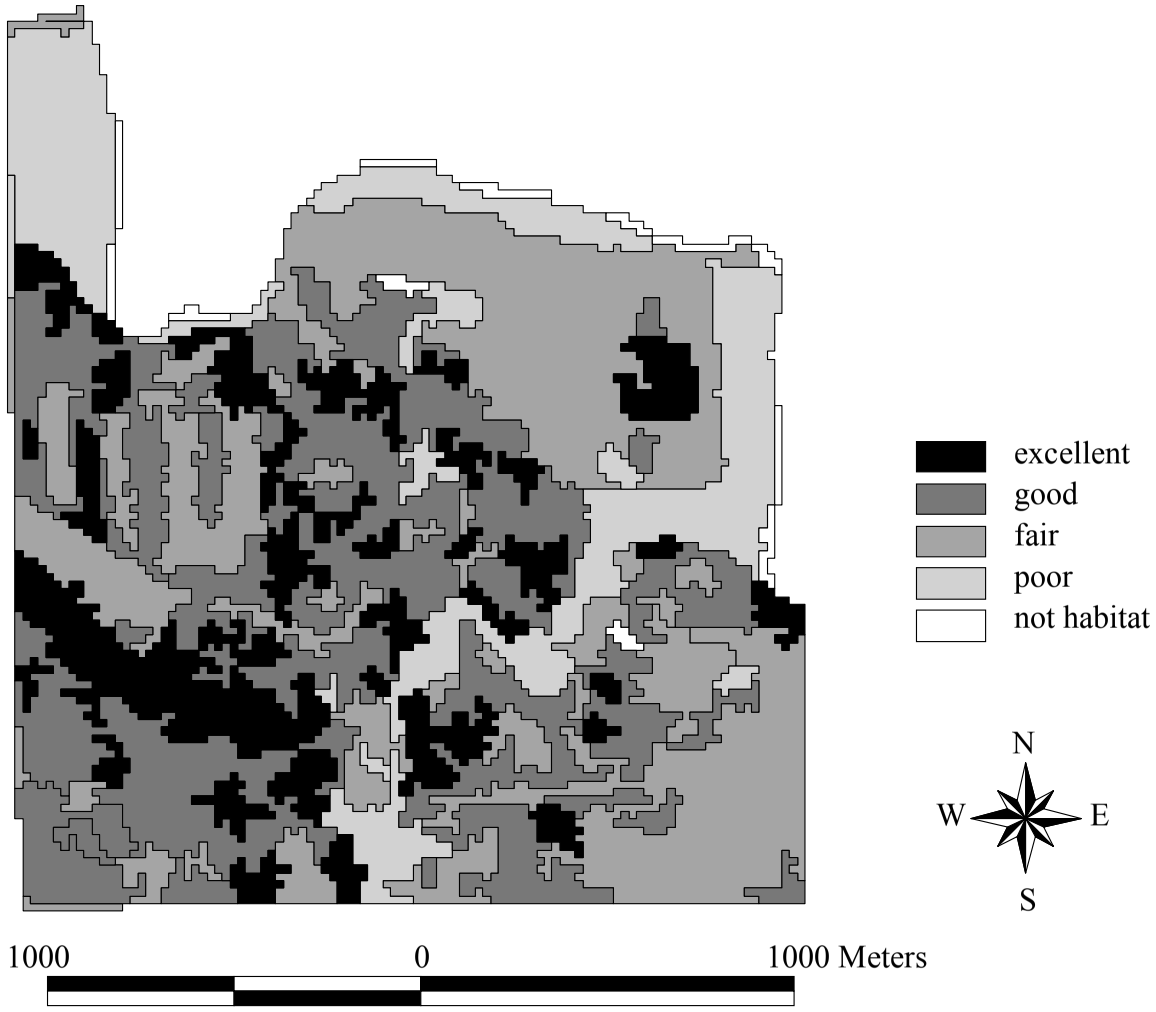



Figure 12. Pollen classification for March and April.

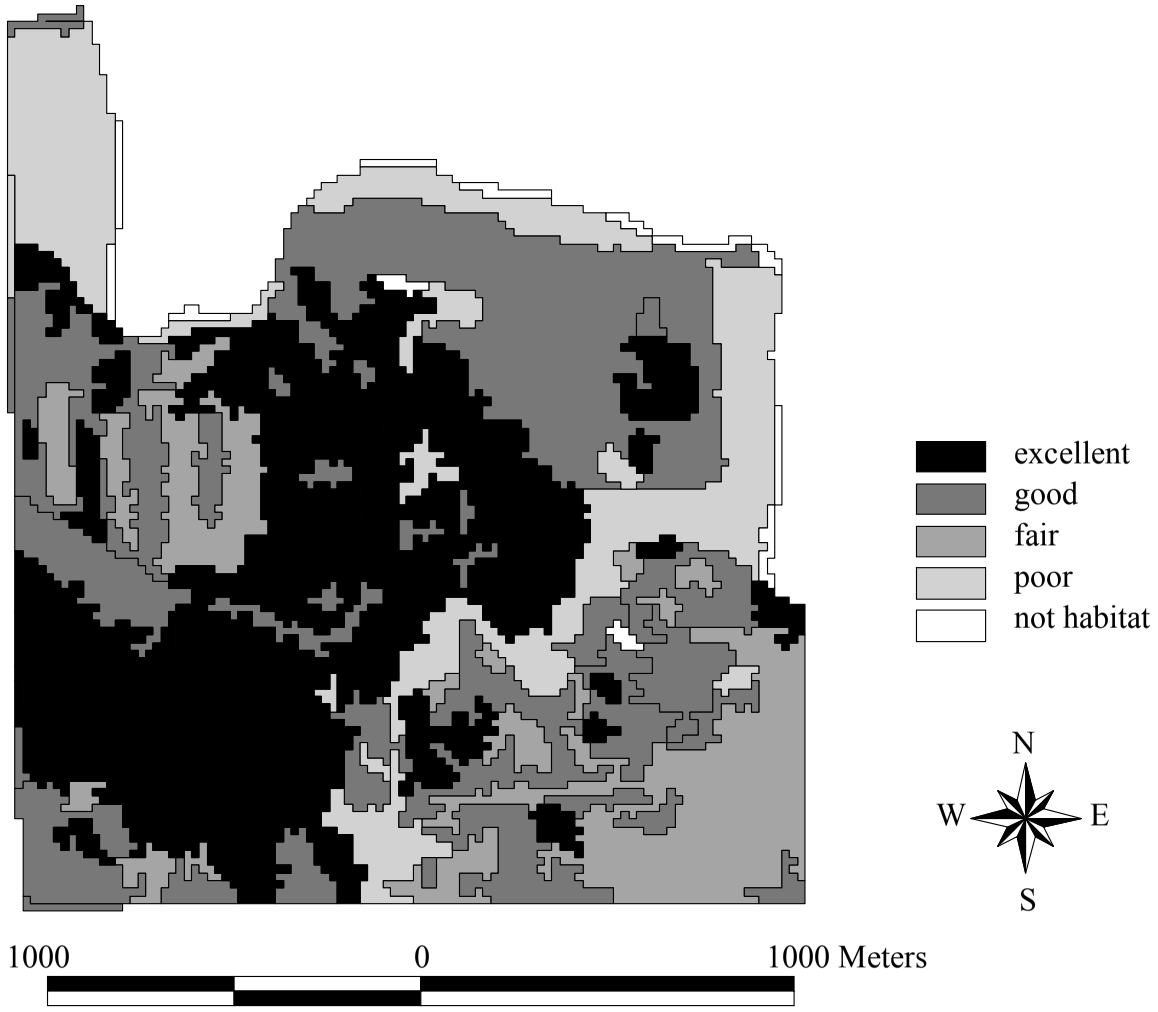



Figure 13. Pollen classification for May through September.

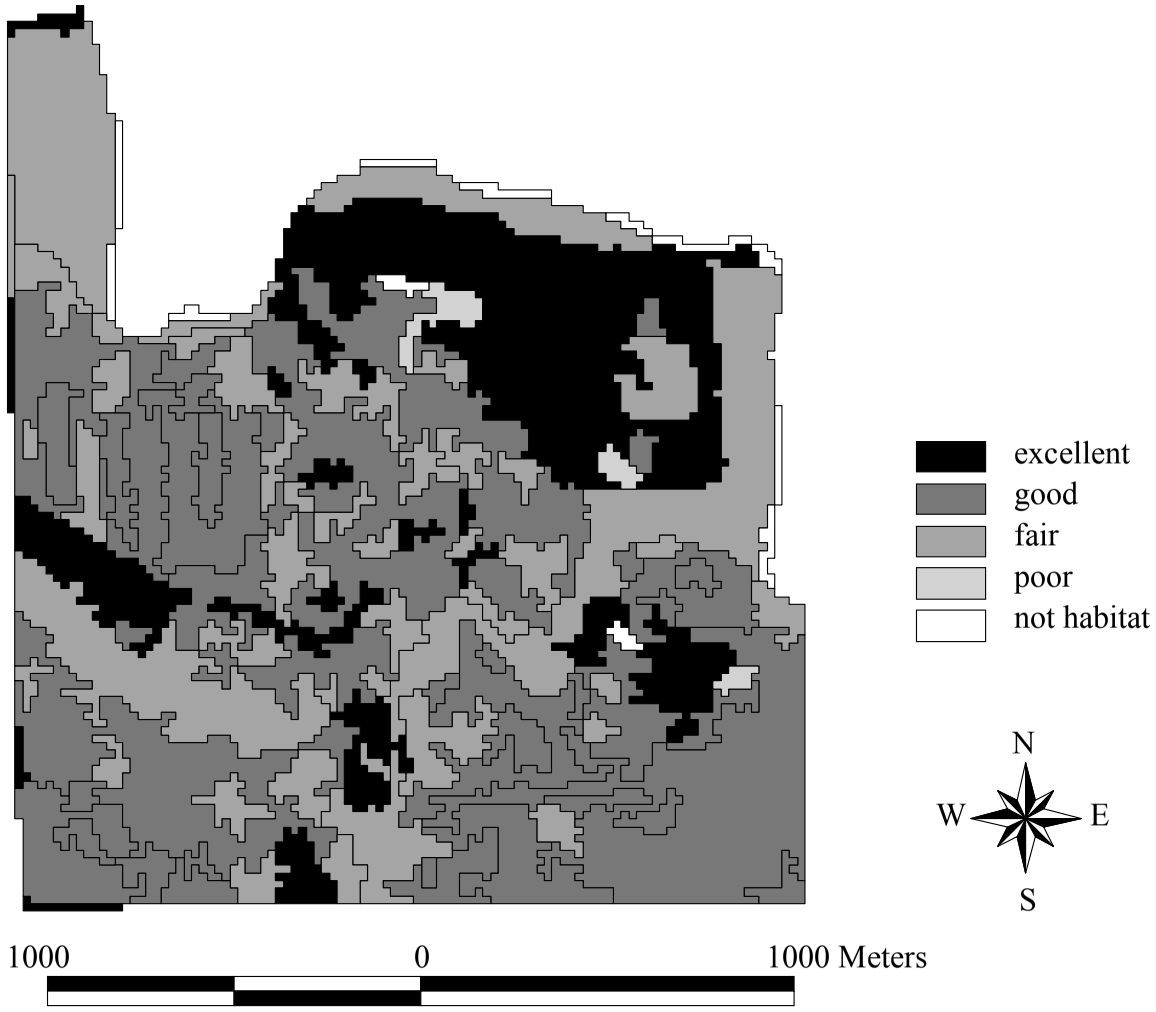



Figure 14. Pollen classification for October.

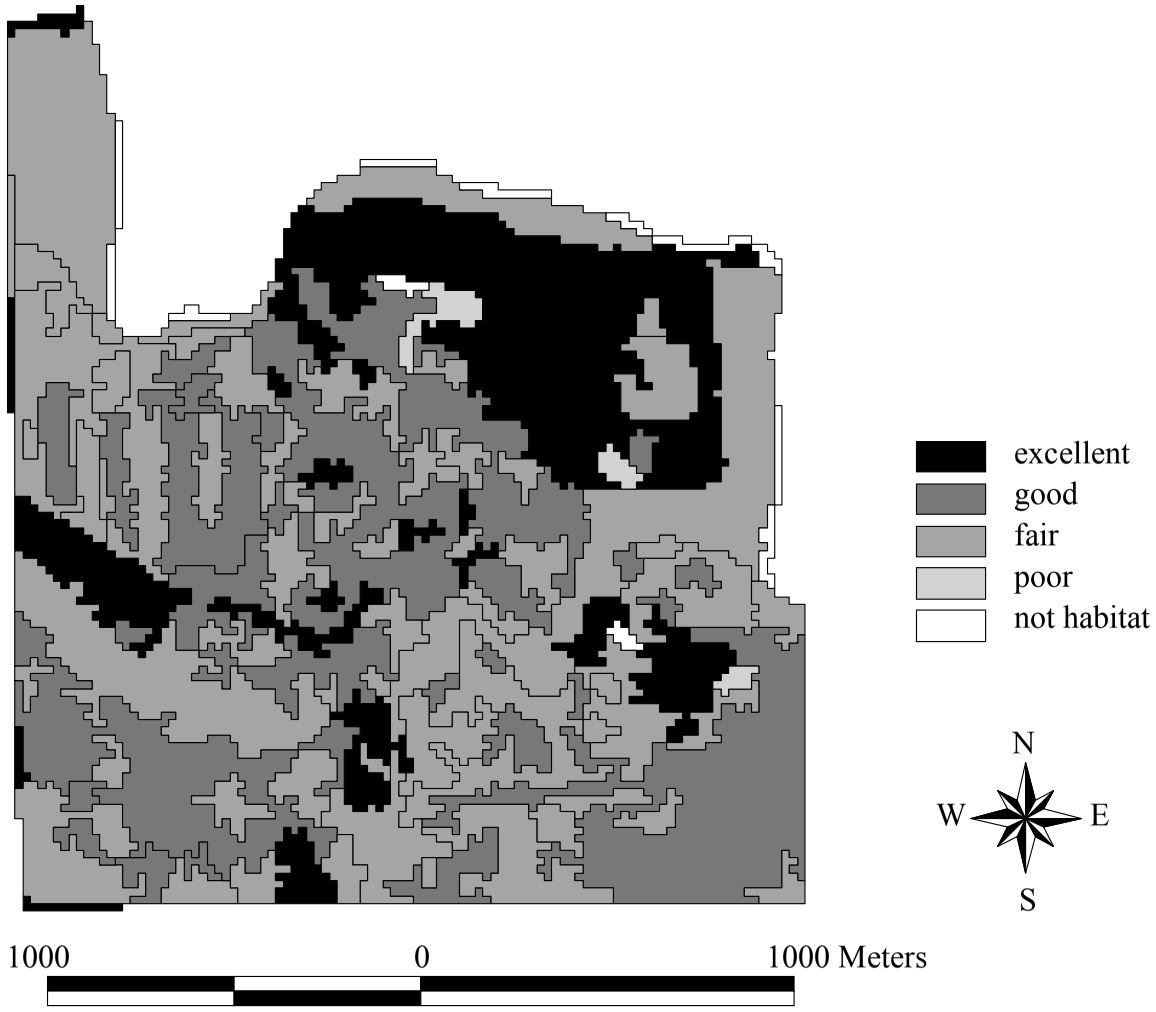



Figure 15. Pollen classification for November.

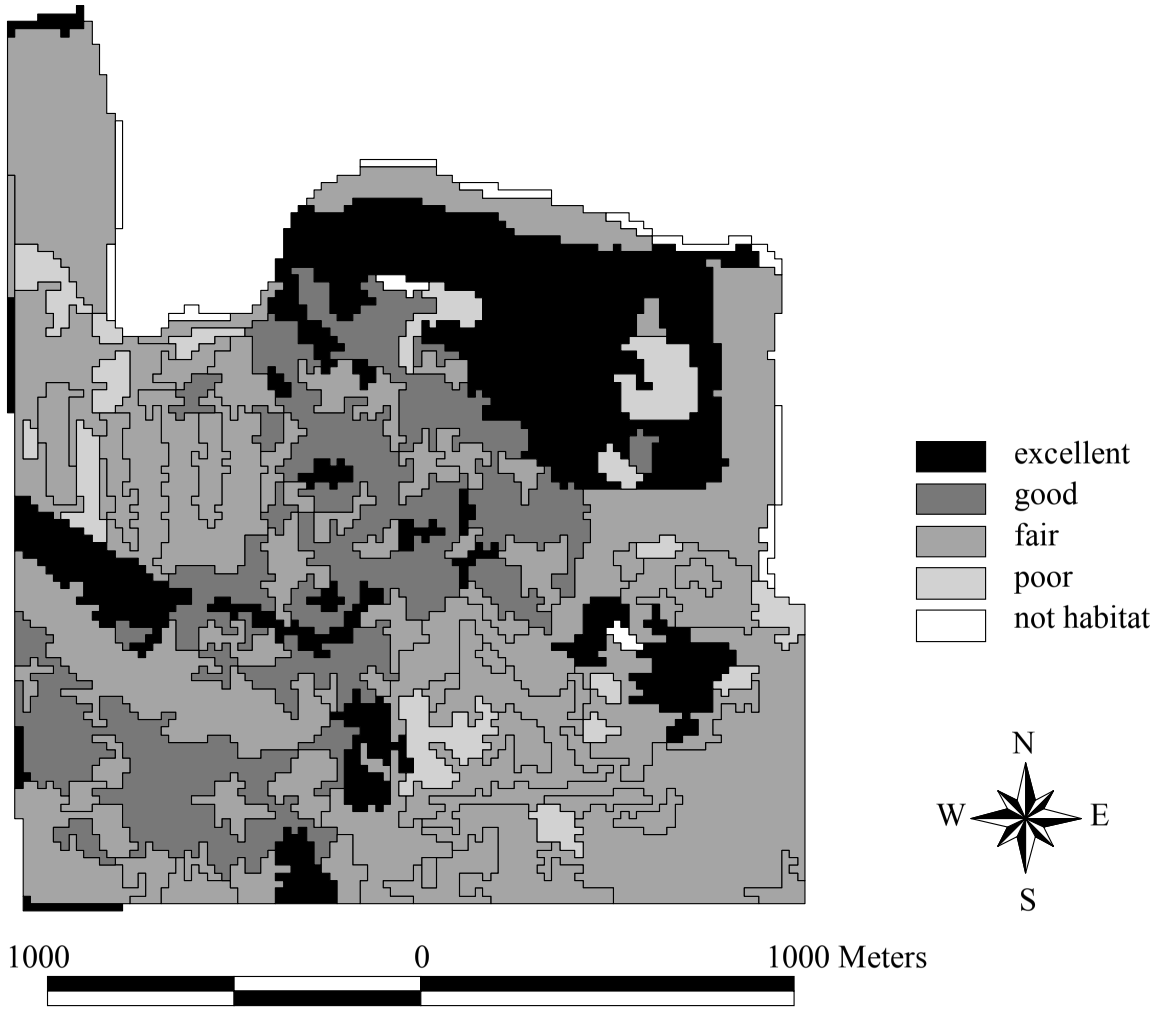



Figure 16. Pollen classification for December.

Table 11. Conservative estimates of the kg of pollen produced in the study site, as well as the kg of pollen produced within the cumulative potential foraging range of all the colonies in the study site.

| | study site only | expanded foraging area (study site + 66.79 %) |
|---|---|---|
| month | kg pollen | kg pollen |
| Jan | 192 | 321 |
| Feb | 672 | 1121 |
| Mar | 3431 | 5723 |
| Apr | 3183 | 5309 |
| May | 3221 | 5372 |
| Jun | 2969 | 4951 |
| Jul | 2956 | 4931 |
| Aug | 3012 | 5025 |
| Sep | 3067 | 5116 |
| Oct | 948 | 1582 |
| Nov | 886 | 1478 |
| Dec | 366 | 610 |
| annual | 24905 | 41538 |
| colonies supported | 1895 | 3161 |

Table 12. Overall classifications for nectar, pollen, and cavity availability and the combined classification for resource availability by habitat type. Shading emphasizes the availability rankings from lightest (poor) to darkest (excellent). Rankings of "not habitat" are represented by a dashed cell border.

| resource | aquatic plants | brushland (dense) | brushland (open) | brushland-grassland | disturbed | grassland | live oak (dense) | live oak (open) | woodland |
|---|---|---|---|---|---|---|---|---|---|
| nectar | poor | excellent | good | fair | poor | fair | excellent | good | poor |
| pollen | poor | excellent | good | fair | poor | good | excellent | excellent | poor |
| cavities | not habitat | fair | fair | poor | not habitat | poor | excellent | good | good |
| combined | poor | good | good | poor | poor | fair | excellent | good | poor |

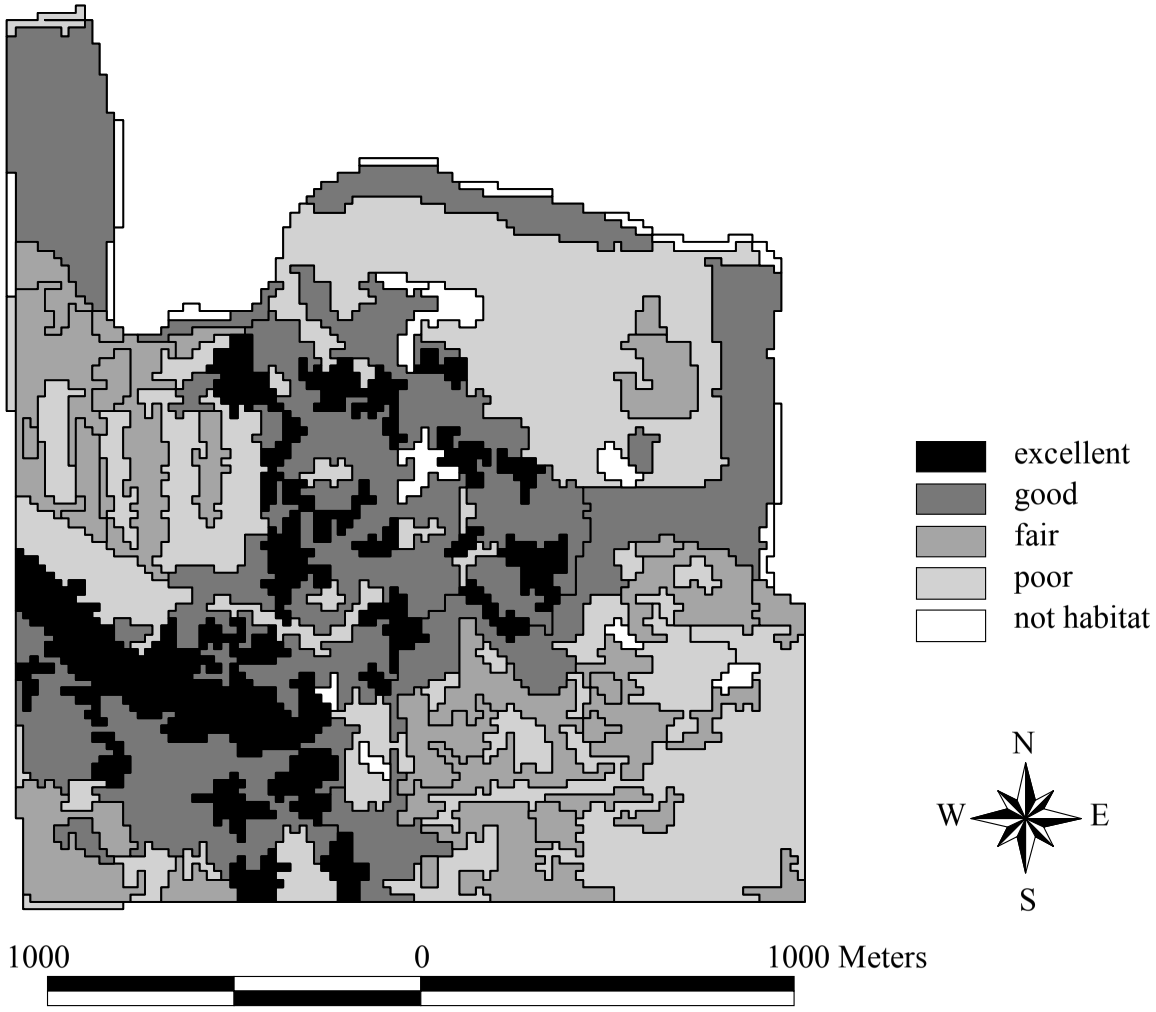



Figure 17. Overall cavity classification.

Combined classification

The overall nectar classification showed that dense live oak and dense brushland were the best sources of nectar, followed by open live oak and open brushland (Table 12, Figure 18). Grassland and brushland-grassland were fair sources, while the woodland, disturbed, and aquatic habitats provided only poor nectar availability (Table 12, Figure 18). Dense live oak, open live oak, and dense brushland were excellent pollen sources, followed by open brushland and grassland as good sources (Table 12, Figure 19). Brushland-grassland was a fair source of pollen, while the woodland, aquatic, and disturbed habitats were poor sources.

The combined classification showed the dense live oak habitat as the best overall habitat for feral honey bee colonies in the study area (Table 12, Figure 20). Open live oak, dense brushland, and open brushland provided good habitat, while grassland was a fair source of the resources important to feral colonies (Table 12, Figure 20).

DISCUSSION

Nectar and pollen availability were highest from March through September and lowest from October through February (Tables 5, 9). Dense live oak provided the most nectar and pollen, suggesting woody species were the main source of nectar and pollen in the live oak group (dense live oak, open live oak, and grassland) (Tables 5, 9). However, open brushland was a better source of nectar and pollen than the dense brushland or brushland-grassland. Therefore, increasing or decreasing the contribution

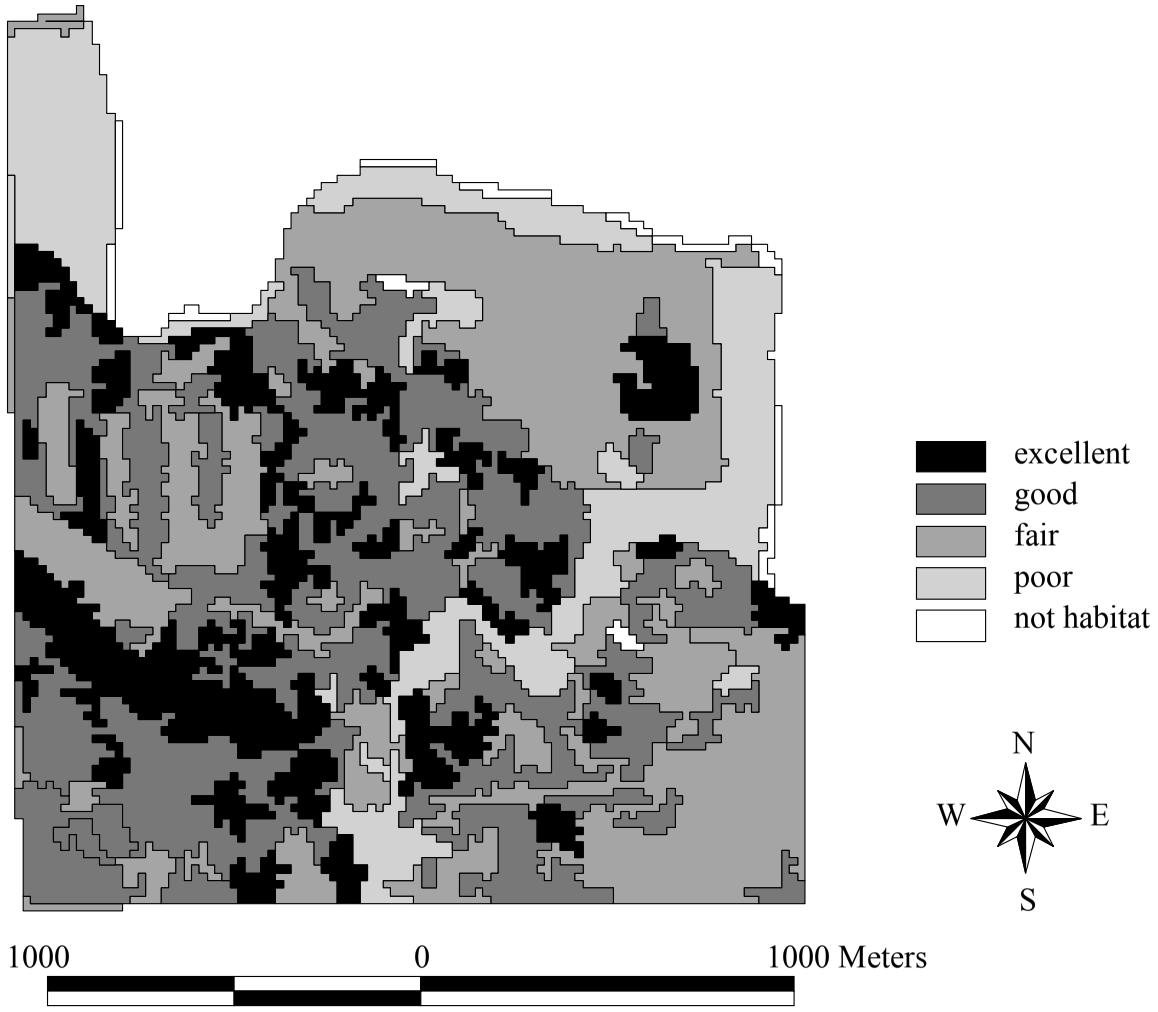



Figure 18. Overall nectar classification.

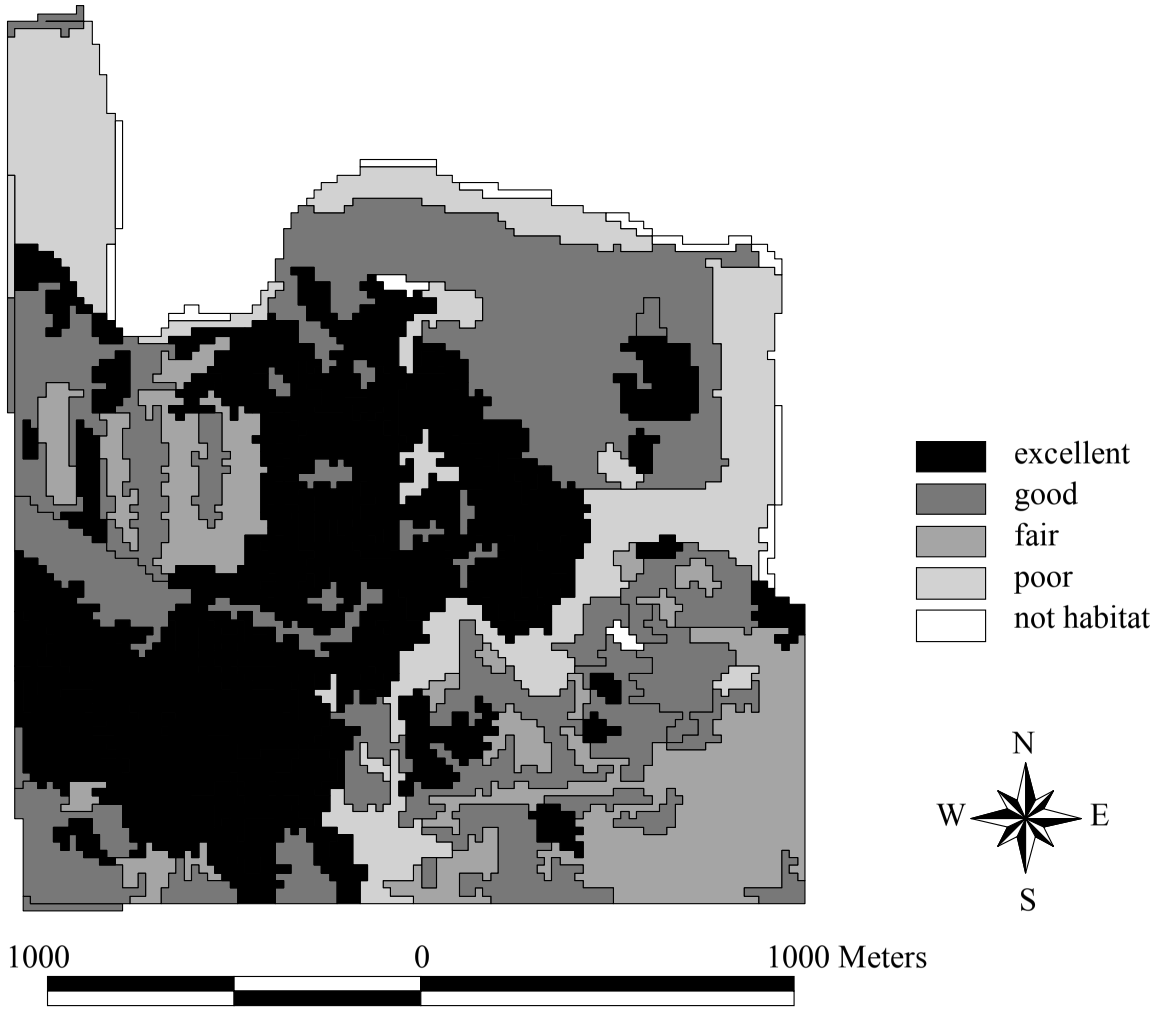



Figure 19. Overall pollen classification.

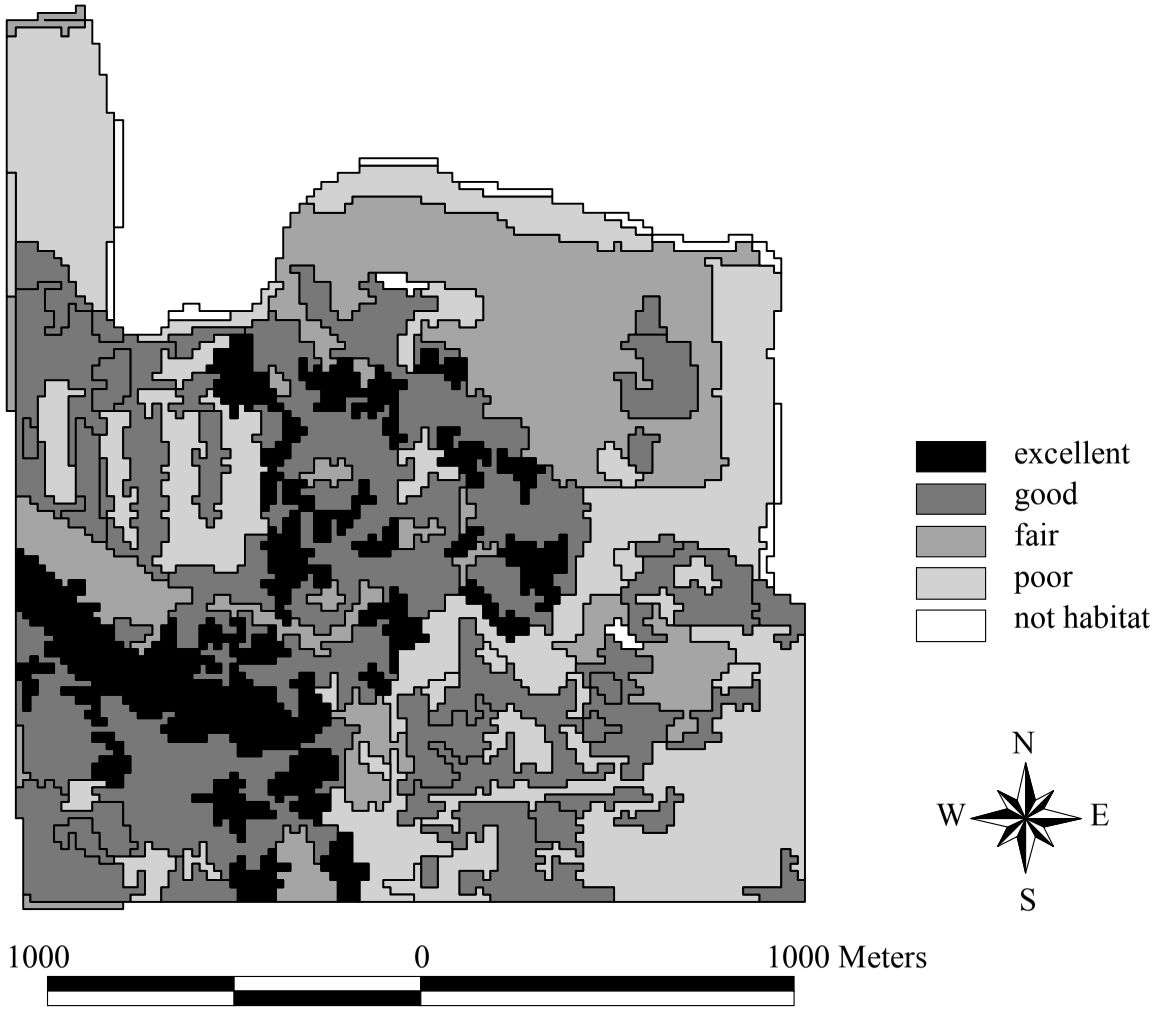



Figure 20. Combined classification.

of forbs or woody species failed to improve nectar or pollen availability in the brushland group.

When examining nectar availability between habitats within a month, the dense habitats (dense live oak and dense brushland) were ranked higher from January through September, suggesting woody species contributed more to nectar availability than forbs during this time period (Table 6). Dense live oak, open live oak, grassland, and woodland received excellent rankings for October and November. However, only open live oak and grassland received excellent rankings in December, indicating that forbs were an important nectar source at the end of the year (Table 6).

Within months, the quality of the brushland group for pollen decreased in value from February through September when the contribution of forbs was increased and the contribution of woody species was decreased (brushland-grassland and open brushland were lower in quality than dense brushland), indicating that woody species were more important for pollen than forbs in the brushland group (Table 10). However, later in the year (October through December), the brushland-grassland was higher in quality than the dense brushland, showing that forbs were important sources of pollen at the end of the year. Similarly, dense live oak tended to be better than open live oak and grassland until later in the year (October through December), when grassland was the best source of pollen (Table 10). Therefore, woody species were important sources of pollen throughout much of the year, while forbs were important at the end of the year.

Dense live oak provided the best source of cavities, followed by open live oak and woodland with good rankings (Table 12). The combined rankings for cavity, nectar,

and pollen sources also showed dense live oak as the best overall source for cavities, pollen, and nectar (Table 12). However, the study area only consisted of 11.3 % dense live oak habitat and dense live oak had the lowest level of interspersion and juxtaposition (Table 3). The low interspersion and juxtaposition value was due to the grouping of the dense live oak habitat within the open live oak habitat. Therefore, the dense live oak habitat consisted of a large number of small patches located mainly within the open live oak habitat.

Based on the combined classification, open live oak, dense brushland, and open brushland were considered good sources of resources important to feral honey bee colonies (Table 12). These habitats made up 18.6 %, 4.9 %, and 13.9 % of the study area, respectively (Table 3). Interspersion and juxtaposition levels were higher than for dense live oak, but lower than for the grassland and woodland habitats. The open live oak habitat contained fewer, but larger patches compared to the dense live oak habitat. Dense brushland patches were typically smaller than open brushland patches and dense live oak patches.

Pollen did not appear to be limiting for the colonies at any time throughout the year (Table 11). Nectar was probably limiting during January and December, and possibly February, October, and November (Table 7). However, honey bee colonies do store pollen and nectar (in the form of honey) in the hive for use during times of low resource availability. Buchmann et al. (1992) also found that nectar availability was lower than pollen availability, estimating that feral colonies in Saguaro National Monument used 1.5 % of the annual pollen production and 7.3 % of the annual nectar

production in the area. Equivalent calculations for this study area, based on 81 colonies (the largest number present throughout the duration of a single year during the 11 year survey period) and the assumption that the colonies met their nutritional needs within the study area, suggest the feral honey bee population uses 4.1 % of the annual pollen production and 32 % of the annual nectar production within the study site. However, Buchmann et al. (1992) estimated 4.0 colonies per $km^2$, while the colony density in the Welder study site was 13.4 colonies per $km^2$ during the 1995 survey period (the period with 81 colonies). Also, the estimates of nectar and pollen production in the Welder study site were intended to be very conservative, and annual production was probably much higher during most years.

Based on the conservative estimates of nectar and pollen availability (Tables 7, 11), more resources were produced than were used by the feral colonies. Other animals may have consumed some of this surplus, but it brings into question whether the study area could support an even larger number of feral colonies. However, temporal patterns of resource availability may be more important than the overall quantity present throughout the year (O'Neal and Waller 1984). For example, the availability of nectar and pollen at critical times of the year, such as when brood rearing begins in the early spring, influences colony growth and reproduction. In some areas, such as desert regions, resources may be abundant only during brief pulses of flowering (Buchmann et al. 1992). Therefore, the foraging range of colonies expands and contracts with resource availability, with reports of honey bees foraging anywhere from a few meters to over 10000 m from the hive (Visscher and Seeley 1982).

The nectar and pollen production values developed for this study were based on conservative estimates of flowering throughout the blooming period of a plant. However, many plants would not bloom throughout their entire reported flowering period, but would flower in pulses based on environmental conditions. Although the overall estimates of nectar and pollen production were very conservative, they were averaged over the flowering period of a plant to reflect baseline conditions. The actual flowering of these plants, and thus the availability of nectar and pollen, would vary within and between years depending on factors such as temperature and precipitation. Therefore, pulses and dearths in resource availability are not reflected in the monthly estimates, and temporal patterns of high and low resource abundance have important implications for colony growth and reproduction.

At the spatial scale of the study site, no habitat type served the function of the matrix, in terms of large area covered, high connectivity, and control over landscape dynamics (Forman 1995). At a broader spatial extent, the brushland habitat clearly met all three criteria. The encroachment of the brushland into the grassland habitat has been well documented in southern Texas, as well as other areas (Archer 1995). The general temporal sequence involves the invasion and establishment of *Prosopis glandulosa*, after which soils become enriched with $N_2$ and suitable for other woody species, such as *Zanthozylum fagara*, *Berberis trifoliata*, *Diospyros texana*, and *Celtis pallida* (Archer et al. 1988, Barnes and Archer 1996). The establishment of these other woody species eventually leads to the loss of *P. glandulosa* and prevents it from re-colonizing the area. Therefore, the response of these species to subsequent changes due to the loss of *P.*

*glandulosa* will shape the resulting structure and function of this woody community (Archer 1995).

In relation to honey bee biology, the brushland habitat is a poor source of cavities, but a relatively good source of pollen and nectar. *P. glandulosa*, as well as *Z. fagara*, *B. trifoliata*, *D. texana*, and *C. pallida*, are important nectar sources in the brushland community (Table 4), while *P. glandulosa*, *B. trifoliata*, and *C. pallida* are also important pollen sources (Table 8). However, the grassland provides pollen from October through December when pollen availability is very low. The grassland is also an important source of nectar at that time, although the dense live oak, open live oak, and woodland habitats also provide nectar. Therefore, the encroachment of brushland species into grassland habitats may decrease the nectar and pollen available to the feral colonies at a critical time of the year.

Cavities restrict the location of colonies to habitats with larger trees, although colonies do build nests in human made structures or occasionally in exposed locations. Depending on the spatial configuration of the habitats with cavities (mainly dense live oak, open live oak, and woodland) in relation to the encroaching brushland habitat, the impact of the decline in grassland habitat could be intensified. The feral colonies could be forced to forage at greater distances and/or collect less suitable pollen types. Therefore, the expansion of the brushland and contraction of the grassland could impact the suitability of the Welder Wildlife Refuge for feral honey bees.

Although cavities are abundant in the study area, cavities may be limiting at a broader spatial extent. Based on vegetation communities defined by McMahan et al.

(1984), cavities are mainly available in the mesquite-live oak-bluewood parks in the western one-quarter of the Welder Wildlife Refuge (Figure 21). Within San Patricio County, cavities are probably also available in the live oak woods/parks (Figure 21). Together, the mesquite-live oak-bluewood parks and live oak woods/parks comprise 13 % of San Patricio County. In terms of the Texas coastal bend (including the counties of Gonzales, Lavaca, Dewitt, Victoria, Jackson, Goliad, Calhoun, McMullen, Live Oak, Bee, Refugio, Aransas, San Patricio, Duval, Jim Wells, Nueces, Kleberg, Brooks, and Kenedy), live oaks are restricted to mesquite-live oak-bluewood parks, live oak woods/parks, post oak woods, forest and grassland mosaic, and post oak woods/forest, which comprise 22 % of the area (Figure 22). The highly suitable habitat of mesquite-live oak-bluewood parks found on the Welder Wildlife Refuge makes up only 2 % of the Texas coastal bend (Figure 22).

The distribution and abundance of nectar and pollen sources also varies at different scales. Cropland comprises 77 % and 33 % of San Patricio County and the Texas coastal bend, respectively. Pollen from crop species was collected in very small amounts on the Welder Wildlife Refuge (unpublished data, K. A. Baum, W. L. Rubink, and R. N. Coulson). In San Patricio County and the Texas coastal bend, cotton and sorghum are important crops (Texas Agricultural Statistics Service 2001). Cotton provides variable amounts of nectar and sorghum provides pollen and honey dew (Pellett 1977). Mesquite-blackbrush brush and mesquite-granjeno parks contribute an additional 31 % to the Texas coastal bend, and are probably good sources of nectar and pollen. Therefore, nectar and pollen sources are abundant within the study area and on the

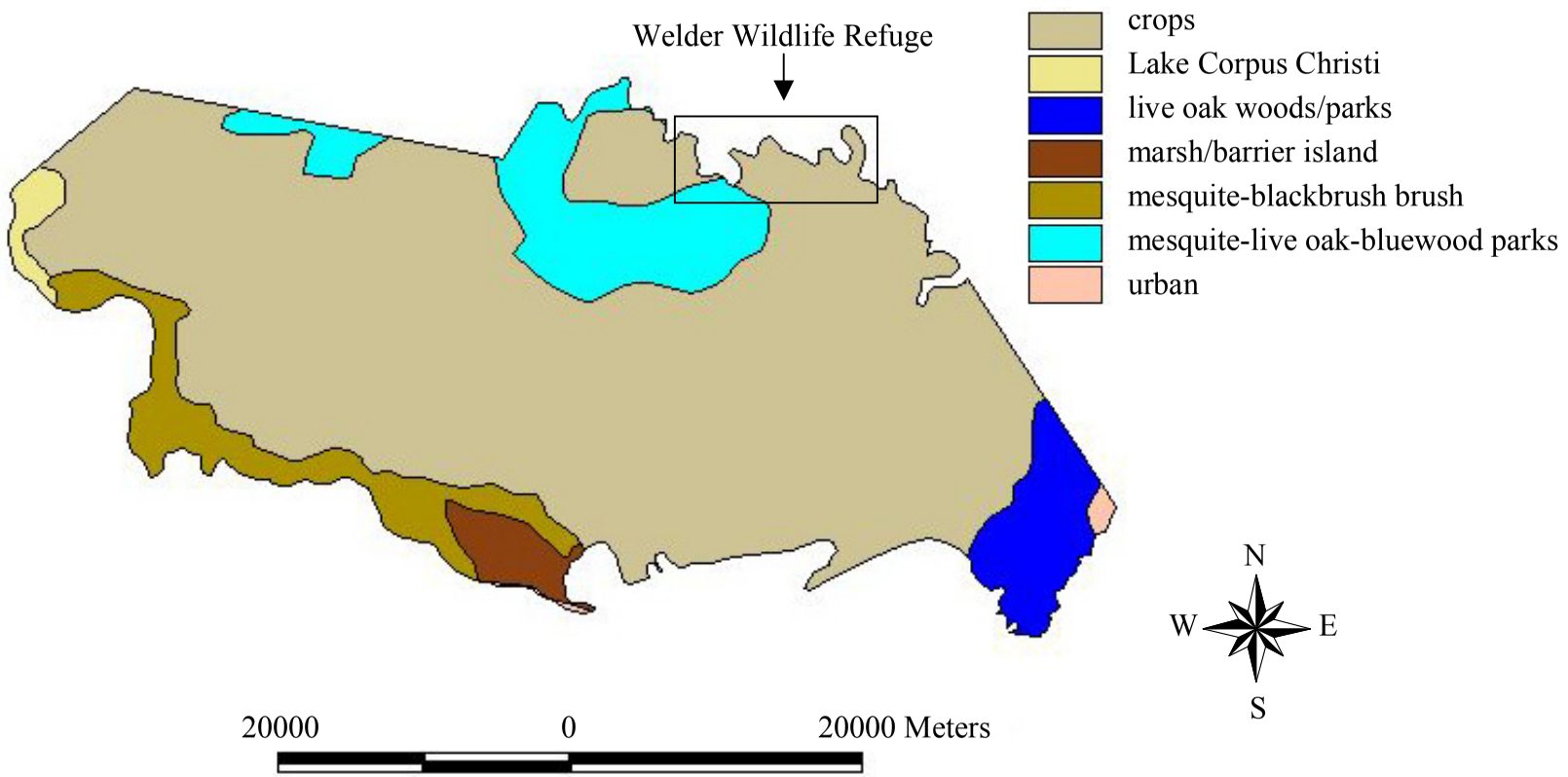



Figure 21. Vegetation classification for San Patricio County, Texas and the Welder Wildlife Refuge from McMahan et al. (1984).

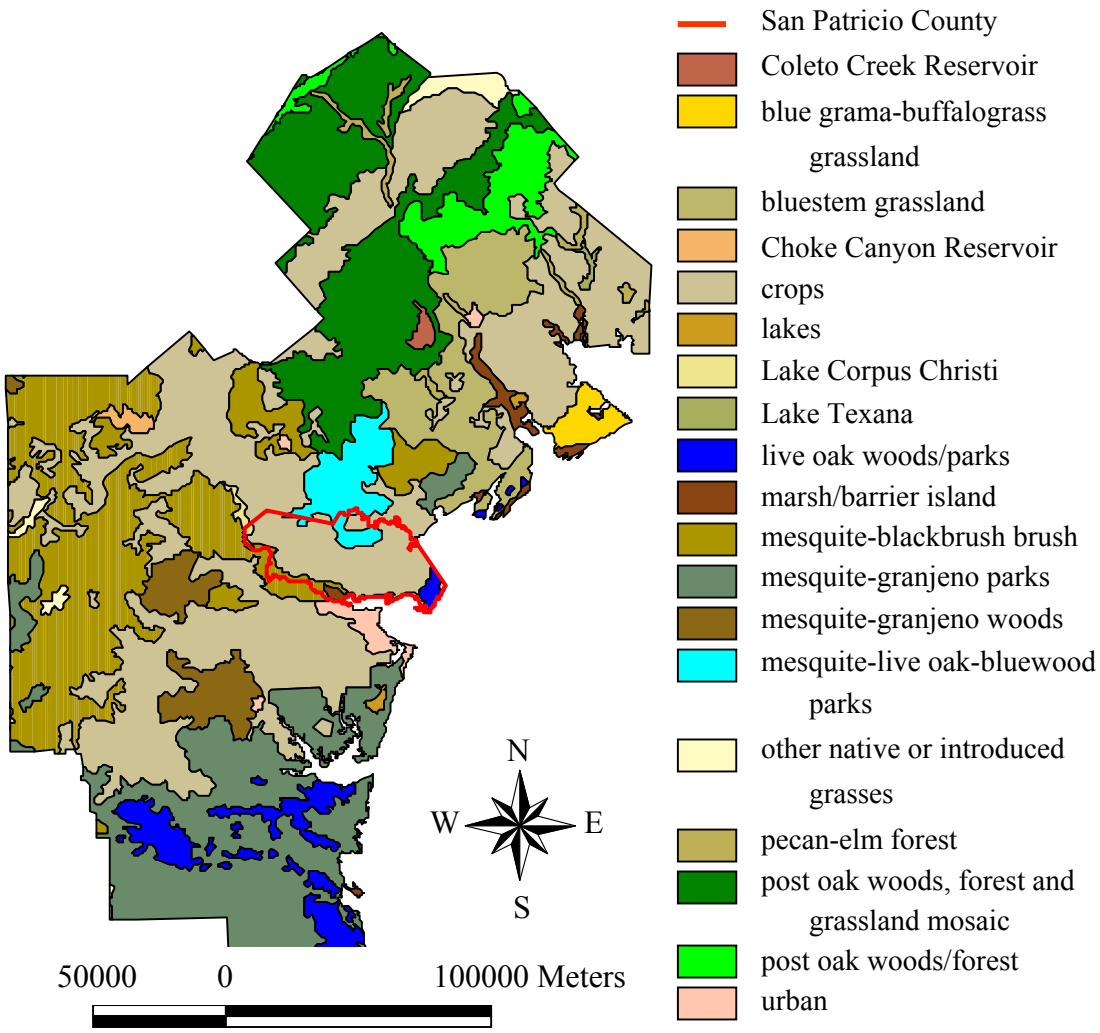



Figure 22. Vegetation classification for counties in the Texas coastal bend from McMahan et al. (1984).

Welder Wildlife Refuge, less abundant at the scale of San Patricio County, and moderately abundant within the Texas coastal bend.

In conclusion, the combined rankings for cavity, nectar, and pollen sources showed that the dense live oak habitat was the best overall source for cavities, pollen, and nectar. Resources appeared not to be limiting to feral honey bees in the study area, except during December and January. However, the distribution and abundance of cavity, nectar, and pollen sources varied at different spatial scales. Cavity, nectar and pollen sources were abundant within the study area and on the Welder Wildlife Refuge, less abundant within San Patricio County, and moderately abundant within the Texas coastal bend ecoregion.

The approach of defining a rule base for feral honey bees in a coastal prairie landscape based on landscape classifications of important resources could be applied to other organisms and landscapes. If less information is available to develop the classifications, the rule base could incorporate many of the decisions included in the classifications developed for this study. NetWeaver™ and GeoNetWeaver™ provide a useful framework for developing a rule base and implementing it spatially using landscape classifications.

# CHAPTER III

# DISTRIBUTION AND ABUNDANCE

## INTRODUCTION

The location of nest sites, as well as structural and environmental characteristics of nest sites, influence the survival, growth, and reproduction of feral honey bee (*Apis mellifera*) colonies (Seeley 1985, Ratnieks and Nowakowski 1989). Cavity volume constrains the amount of brood production and food storage, and limits the number of adults in a colony (Seeley 1985, Winston 1987). Entrance size and orientation influence the thermoregulation of the colony (Szabo 1983), while entrance size, height, and number influence a colony's ability to defend the nest against predators (Seeley 1985). Other factors, such as cavity exposure and visibility, also influence cavity quality for feral colonies (Winston 1987). Cavity selection by feral colonies has important implications for the dispersal of Africanized honey bees. Understanding nest site selection and the population ecology of feral colonies also provides insight into how Africanized honey bees will impact agricultural and beekeeping practices (McNally and Schneider 1996).

Africanized honey bees, hybrids between European and African (*A. m. scutellata*) honey bees first arrived in the United States in 1990 (Hunter et al. 1993, Rubink et al. 1996). For the purposes of this study, European refers to the existing honey bee population in the United States before the arrival of Africanized honey bees.

The background population consisted of a variety of subspecies of *A. mellifera*, mostly from Europe (Sheppard 1989a, 1989b).

A number of studies have identified and described the locations of feral honey bee colonies (Seeley and Morse 1976, Avitabile et al. 1978, Taber 1979, Visscher and Seeley 1982, Boreham and Roubik 1987, Schneider and Blyther 1988, Wenner 1989, Gambino et al. 1990, Morse et al. 1990, Schneider 1990, Ratnieks et al. 1991, Oldroyd et al. 1994, Oldroyd et al. 1995, McNally and Schneider 1996). Many have simply estimated the density of feral colonies, while others have examined structural attributes of nest sites. However, few studies have evaluated spatial patterns of cavity use (Oldroyd et al. 1995, McNally and Schneider 1996) and no studies have evaluated spatial patterns through time.

The goal of this study was to evaluate spatial and temporal patterns in the distribution and abundance of feral colonies on the Welder Wildlife Refuge by examining nest site characteristics, population trends, and cavity use. Specific objectives were 1) to compare the density of feral colonies in this study with densities reported in the literature, 2) to evaluate cavity suitability for feral honey bees based on structural and environmental attributes of the cavities, 3) to compare the structural and environmental attributes of cavities occupied only by Africanized or only by European colonies, 4) to examine spatial and temporal patterns in cavity use by the feral colonies, and 5) to examine the spatial and temporal distribution of Africanized and European colonies.

# METHODS

## Study site description

The study site was located on the Welder Wildlife Refuge in San Patricio County, Texas. It consisted of a mosaic of four main habitat types, including brushland, live oak, grassland, and woodland habitats (unpublished data, Welder Wildlife Foundation). The brushland community is dominated by blackbrush acacia (*Acacia rigidula* G. Bentham), agarito (*Berberis trifoliata* M. Moricand), and honey mesquite (*Prosopis glandulosa* J. Torrey var. *glandulosa*). Dominant forbs include western ragweed (*Ambrosia psilostachya* A. P. de Candolle), spiny aster (*Chloracantha spinosa* (G. Bentham) G. Nesom var. *spinosa*), and upright prairie coneflower (*Ratibida columnifera* (T. Nuttall) E. Wooton and P. Standley). The predominant woody species in the live oak community is live oak (*Quercus virginiana* P. Miller), while huisache (*Acacia minuata* (M. E. Jones) P. de Beauchamp subsp. *minuata*), blackbrush acacia, agarito, woolybucket bumelia (*Sideroxylon lanuginosum* A. Michaux), hog plum (*Colubrina texensis* (J. Torrey and A. Gray) A. Gray var. *texensis*), Texas kidneywood (*Eysenhardtia texana* G. Scheele), honey mesquite, and lime pricklyash (*Zanthoxylum fagara* (C. Linnaeus) C. Sargent) are of secondary importance. Important forbs include spiny aster, huisache daisy (*Amblyolepis setigera* A. P. de Candolle), plains coreopsis (*Coreopsis tinctoria* T. Nuttall var. *tinctoria*), slender croptilon (*Croptilon divaricatum* (T. Nuttall) C. Rafinesque-Schmaltz), wooly croton (*Croton capitatus* A. Michaux), Texas croton (*Croton texensis* (J. Klotzch) J. Müller of Aargau var. *texensis*), wild gourd (*Cucurbita texana* A. Gray), and silverleaf sunflower (*Helianthus argophyllus* J. Torrey

and A. Gray). The grassland community consists of the same forbs as described for the live oak habitat, but with very few woody plants. Woody species in the woodland habitat include huisache, netleaf hackberry (*Celtis laevigata* C. von Willdenow var. *reticulata* (J. Torrey) L. Benson), hog plum, anaqua (*Ehretia anacua* (M. Terán and J. Berlandier) I. M. Johnston), Texas kidneywood, and western soapberry (*Sapindus saponaria* C. Linnaeus var. *drummondii* (W. Hooker and G. Arnott) L. Benson), with mustang grape (*Vitis mustangensis* S. Buckley) draped over many of the trees (unpublished data, Welder Wildlife Foundation).

Colony density

The refuge has been surveyed for feral honey bee colonies by W. L. Rubink since 1993. To date, 109 cavities containing feral colonies at one point in time have been identified. Africanized honey bees were first recorded on the refuge in 1993 (unpublished data, W. L. Rubink).

The density of feral colonies during each year was calculated based on yearly cavity surveys. The presence or absence of feral colonies in the identified cavities was recorded. Cavities were often surveyed multiple times during any given year, so the status of each cavity the first time it was surveyed each year was used. The number of cavities surveyed increased through time as new cavities used by feral honey bee colonies were found. Densities are reported from 1995 through 2000, since 80 % of the cavities surveyed were found by 1995.

Cavity attributes

Most of the feral colonies in the study area were located in tree cavities. Therefore, I collected detailed measurements for each cavity, including tree species, number of entrances, entrance orientation, entrance size, tree dbh, tree height, cavity height, basal area, canopy closure, ground cover, habitat type, and motte size. Tree species and the number of entrances were obtained by visual inspection. Entrance orientation was recorded using a compass. The width and height of each cavity entrance was measured with a tape measure or estimated in cm when it was not possible to reach the cavity. Entrance width and height were then converted into entrance area based on the area of an ellipse. Diameter at breast height was calculated in cm using a Spencer® Original LoggersTape®. Tree height was recorded in m using a Suunto clinometer with 15 and 20 m scales, while cavity height was measured in m from ground level using a tape measure or visually estimated for high cavities. Basal area was obtained using the five-factor option of a JIM-GEM® Cruz-All. Canopy closure and ground cover (separated into monocot and dicot) were estimated at 10 m from the cavity tree in the four Cardinal directions. Estimates of percent cover were made by looking through a 5 cm diameter by 10.5 cm long hollow tube divided into four quadrants. Percent canopy closure and ground cover were averaged across all directions to obtain an overall value for each cavity tree. Habitat type was identified from a landscape classification of the study area based on vegetation communities (unpublished data, K. A. Baum, W. L. Rubink, and R. N. Coulson), and motte size was obtained from a spatial database of the study area with a resolution of 0.25 m. Mottes are clusters of woody vegetation that

form around a nucleus (in this case a live oak tree) and may eventually expand and coalesce into a contiguous area of woody vegetation. The boundaries of each live oak motte were digitized in ArcView® GIS 3.2 and the area of the resulting polygons calculated using an extension (area calculation for polygon) in ArcView® GIS 3.2. Motte size was only measured for live oaks, because none of the other cavity tree species formed distinct mottes.

Cavity characteristics were evaluated in terms of time occupied and turnover indices calculated for each cavity. Time occupied refers to the proportion of the time surveyed that a cavity was active (contained a colony), while turnover reflects the number of changes in cavity status from active to inactive (did not contain a colony) or inactive to active on consecutive surveys. Therefore, these indices provide an estimate of cavity quality based on honey bee use. Only cavities identified by 1995 were included in the analyses because the values for cavities surveyed only a few times may be biased and 80 % of cavities had been found by then. For example, a cavity surveyed only once (first identified during the most recent survey) would be occupied 100 % of the time with 0 % turnover. I used a Spearman rank correlation coefficient to identify cavity characteristics correlated with the time occupied and turnover indices. I used a chi-square test applied to circular distributions (Batschelet 1965) to examine if there were any patterns in entrance orientation, using eight groups at equal intervals. I used a Kruskal-Wallis test to compare the time occupied and turnover indices between habitat types. I used a Mann-Whitney *U* test to compare cavity characteristics between cavities used only by Africanized and only by European honey bee colonies.

Spatial and temporal patterns

The spatial coordinates for each cavity tree used by feral colonies during the past 12 years were recorded to a submeter accuracy using a Trimble GPS Pathfinder™ receiver and TSC1™ Asset Surveyor™ data logger. When cavities were located in areas with dense canopy cover, an Advantage® Laser Rangefinder was used to calculate the offset from the cavity to where spatial coordinates were obtained.

I used a nearest neighbor analysis to compare observed patterns of cavity occupancy with those expected by chance. The nearest neighbor index (NNI) was calculated by comparing the mean observed nearest neighbor distance with the mean expected nearest neighbor distance for spatially random points (Clark and Evans 1954). I used CrimeStat® v. 2.0 (Levine 2002) for the calculations. The observed nearest neighbor distance (d(NN)) was calculated as

$$d(NN) = \frac{1}{N}\sum_{i=1}^{N} Min(d_{ij}),$$

where Min (dij) was the minimum distance between each point and all other points (its nearest neighbor) and N was the sample size. The mean expected random nearest neighbor distance (d(ran)) was calculated as

$$d(ran) = 0.5\sqrt{\left[\frac{A}{N}\right]},$$

where A was the area of the study site and N was the sample size. In this case, A was defined as the rectangle bounded by the minimum and maximum x and y points. Based on these calculations, the nearest neighbor index (NNI) was

$$NNI = \frac{d(NN)}{d(ran)}.$$

Values close to 1.0 indicate observed average distances do not differ from random, while values less than 1.0 indicate aggregation and values greater than 1.0 indicate dispersion. A Z test was used to identify significant values of the NNI.

Spatial and temporal patterns of Africanized and European colonies

Mitochondrial DNA is maternally inherited and does not recombine during sexual reproduction, passing directly from queen to offspring. Samples for mitochondrial DNA analysis were collected from any cavity active at anytime throughout the year. Therefore, sample sizes differ from those used to calculate colony density. Mitochondrial DNA data were provided by M. A. Pinto (unpublished data, M. A. Pinto, W. L. Rubink. J. S. Johnston, and R. N. Coulson). Sections of the cytochrome *b* gene (Crozier et al. 1991) were amplified, digested, electrophoresed, stained, and visualized. Then, the mitochondrial DNA was classified as African (referred to as Africanized throughout this paper) or non-African (referred to as European throughout

this paper). However, as explained by Sheppard and Smith (2000), some honey bee subspecies from North Africa and areas in southern Europe have African mitochondrial DNA, including *A. m. iberica*, *A. m. intermissa*, *A. m. lamarckii*, *A. m. sicula*, and *A. m. ruttneri*. Following the methodology outlined in Sheppard and Smith (2000), Pinto et al. (2003) analyzed the mitochondrial DNA of samples of feral honey bees collected in the southern United States before the arrival of Africanized honey bees. They concluded that African non-A. *m. scutellata* mitochondrial DNA occurred in very low frequency (< 1 %) and thus the characterization of African mitochondrial DNA as belonging to *A. m. scutellata* is reliable. Therefore, I used a nearest neighbor analysis to compare spatial patterns through time of Africanized and European colonies based on mitochondrial DNA.

## RESULTS

### Colony density

Colony density ranged from 4.5 to 12.5 colonies per $km^2$ from 1995 through 2000 (Table 13). Density was lowest in 1997 and highest in 2000.

### Cavity attributes

Cavities used by feral colonies were located in live oak (*Quercus virginiana* P. Miller), hackberry (*Celtis* spp.), anacua (*Ehretia anacua* (M. Terán and J. Berlandier) I. M. Johnston), cedar elm (*Ulmus crassifolia* T. Nuttall), and mulberry (*Morus rubra* C. Linnaeus) trees (Figure 23). Cavity entrance height varied from ground level to 7.6 m

Table 13. Colony density for each year based on a 6.25 $km^2$ study area.

| year | # active cavities | # colonies per $km^2$ |
|---|---|---|
| 1995 | 73 | 11.68 |
| 1996 | 29 | 4.64 |
| 1997 | 28 | 4.48 |
| 1998 | 41 | 6.56 |
| 1999 | 45 | 7.20 |
| 2000 | 78 | 12.48 |

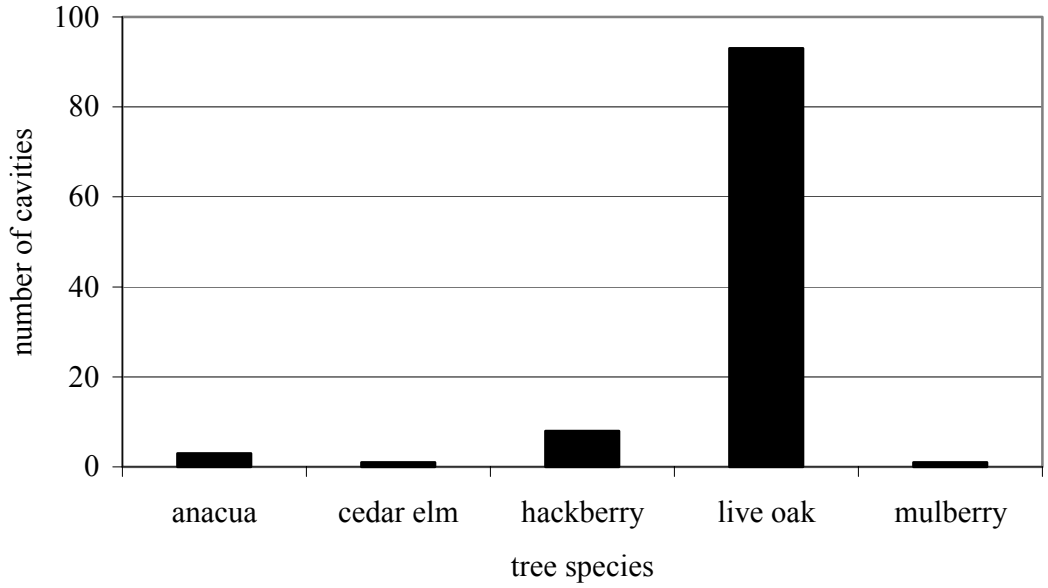



Figure 23. Tree species containing cavities used by feral honey bee colonies on the Welder Wildlife Refuge.

(Table 14). Most cavities only had one entrance, although 12 cavities had two to five entrances. The entrances of cavities used by feral colonies most often faced the northwestern and southeastern directions (Figure 24) and differed significantly from random ($X^2 = 16.92$, df = 7, p-value = 0.0179). Entrance size ranged from 0.8 $cm^2$ to 544.3 $cm^2$, but was typically small (mean = 42.1 $cm^2$). Mean dbh and tree height were 74.96 cm and 11.8 m, respectively. Basal area ranged from 5 to 80 $m^2$/ha, while canopy closure and ground cover ranged from approximately 0 to 90 % (Table 14). The dense live oak habitat contained 56 cavities (51 %), the open live oak habitat contained 36 cavities (33 %), the woodland habitat contained 16 cavities (15 %), and the brushland-grassland habitat contained only one cavity (1 %) (Figure 25). Motte size was variable, with a mean of 351 $m^2$ (Table 14).

Nine cavities were occupied for more than 80 % of the surveys (Figure 26). Five cavities were occupied only during the survey in which they were first found. Turnover was relatively low, ranging from 5 to 30 percent of surveys. However, no cavities were occupied continuously during the surveys (Figure 27).

None of the measured cavity characteristics were significantly correlated with the time occupied or turnover indices (Table 15). None of the measured cavity site characteristics were significantly different between cavities only used by Africanized colonies and only used by European colonies (Table 15).

## Spatial and temporal patterns

Overall, the distribution of all identified cavities used by feral colonies was

Table 14. Descriptive statistics for the measured structural and environmental attributes of cavities occupied by feral honey bee colonies on the Welder Wildlife Refuge.

| | mean ± std dev | median | mode | minimum | maximum | sample size |
|---|---|---|---|---|---|---|
| entrance height (m) | 2.52 ± 1.74 | 2.20 | 2.36 | 0 | 7.6 | 92 |
| number of entrances | 1.15 ± 0.52 | 1.00 | 1.00 | 1 | 5 | 104 |
| entrance size* ($cm^2$) | 42.10 ± 83.29 | 15.71 | 7.07 | 0.8 | 544.3 | 91 |
| dbh (cm) | 74.96 ± 28.13 | 69.00 | 69.00 | 30 | 184.5 | 106 |
| tree height (m) | 11.80 ± 3.34 | 11.00 | 10.00 | 7 | 25 | 106 |
| basal area ($m^2$/ha) | 35.09 ± 15.30 | 35.00 | 40.00 | 5 | 80 | 107 |
| canopy closure (%) | 50.94 ± 22.71 | 55.00 | 68.75 | 0 | 91.3 | 107 |
| ground cover monocot (%) | 28.22 ± 20.31 | 26.25 | 35.00 | 0 | 88.5 | 108 |
| ground cover dicot (%) | 16.03 ± 9.84 | 15.00 | 11.25 | 0 | 48.8 | 108 |
| motte size ($m^2$) | 351.23 ± 370.38 | 237.81 | n/a | 20.2 | 2115.7 | 79 |

* based on the area of an ellipse using entrance width and height

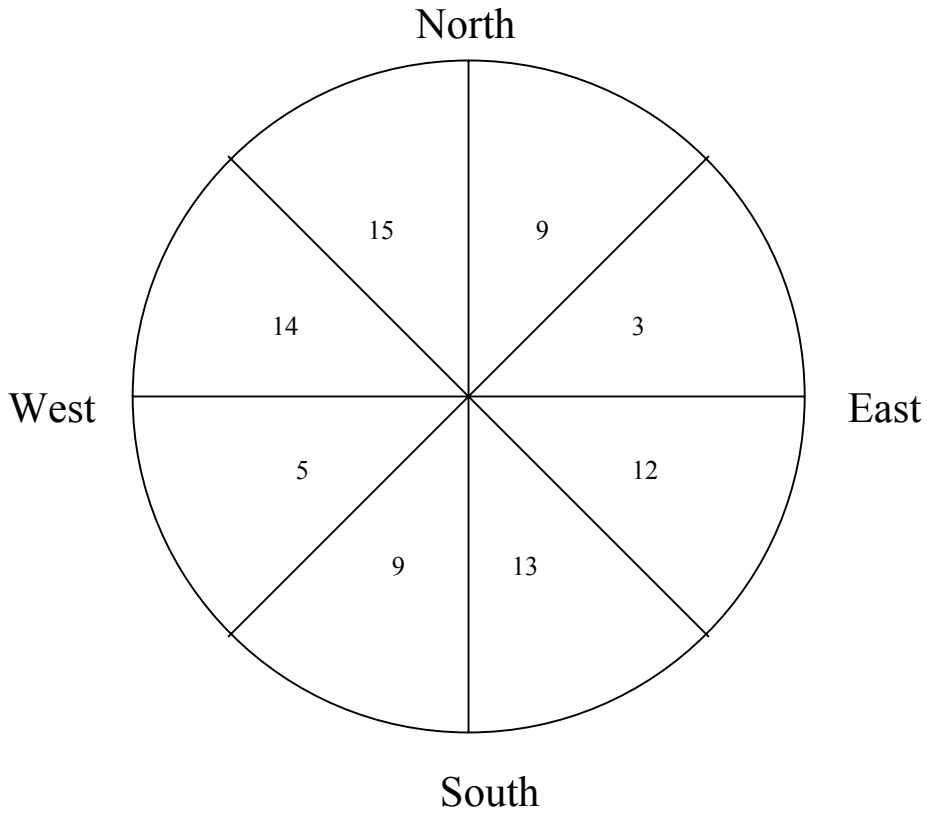



Figure 24. Entrance orientation of cavities used by feral honey bee colonies on the Welder Wildlife Refuge. Numbers represent the number of cavities facing in each direction.

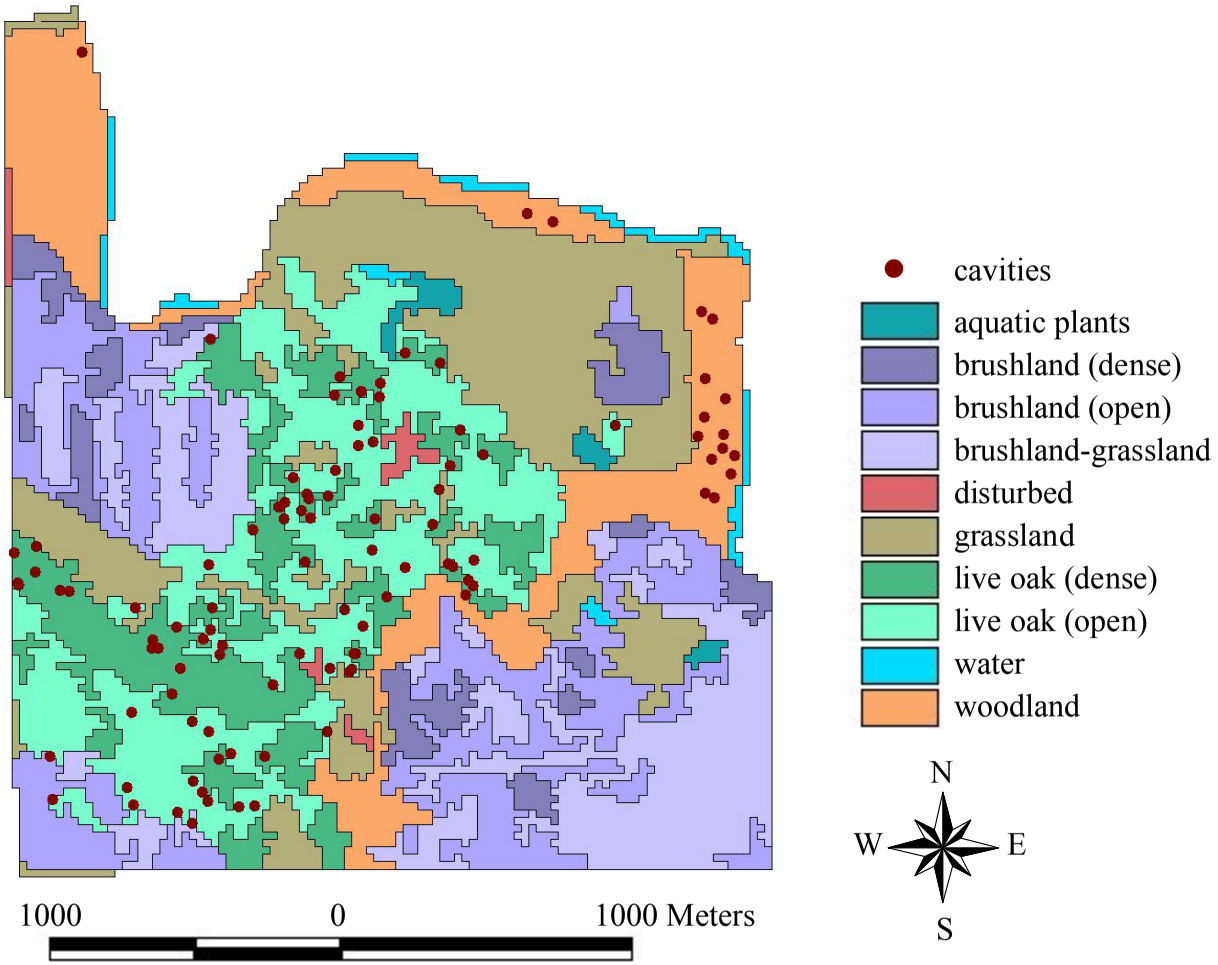



Figure 25. Location of all identified cavities within each habitat type on the Welder Wildlife Refuge.

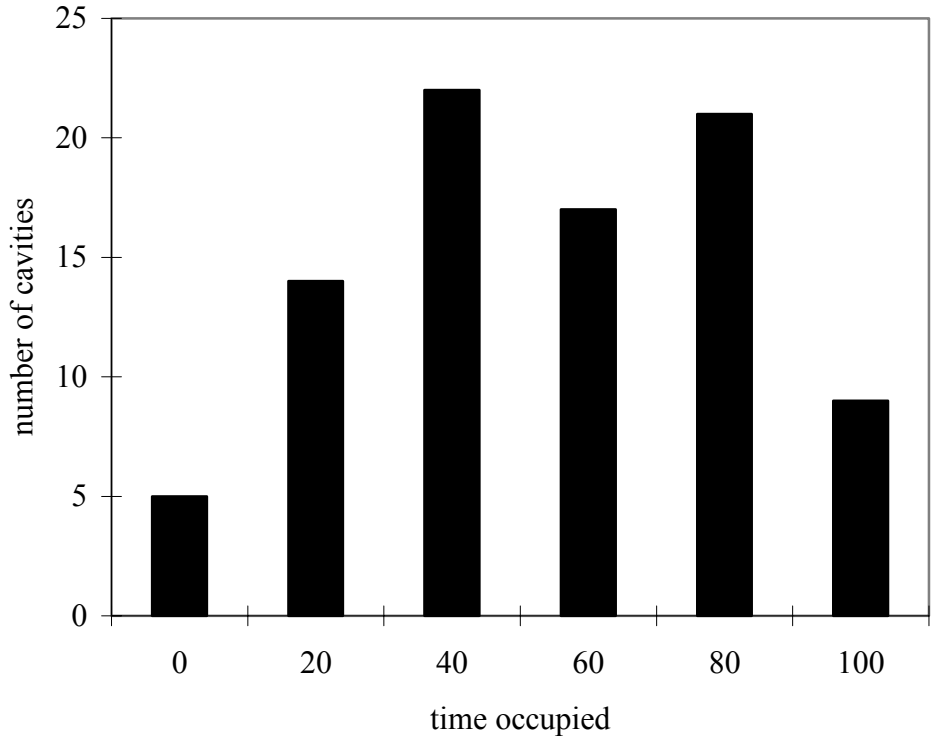



Figure 26. Frequency distribution of the time occupied index for all cavities identified by 1995 on the Welder Wildlife Refuge. Time occupied refers to the proportion of the time surveyed that a cavity was active (contained a colony).

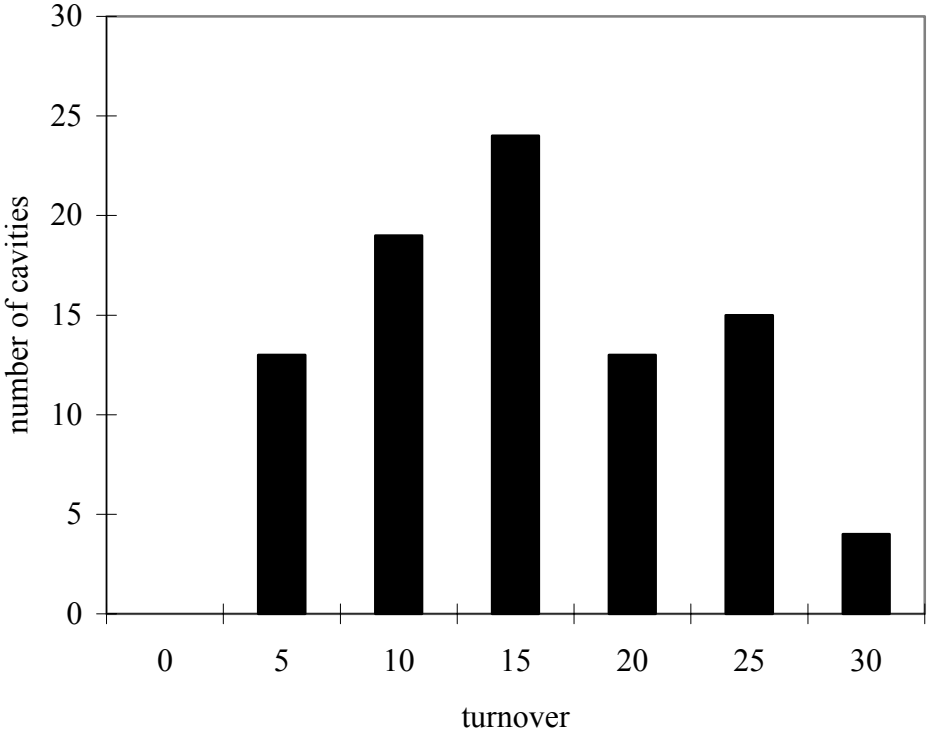



Figure 27. Frequency distribution of the turnover index for all cavities identified by 1995 on the Welder Wildlife Refuge. Turnover reflects the number of changes in cavity status from active (contained a colony) to inactive (did not contain a colony) or inactive to active on consecutive surveys.

Table 15. Results from Spearman correlation tests comparing the structural and environmental cavity attributes and time occupied and turnover indices and Mann-Whitney *U* tests comparing cavities used only by Africanized (AHB) (N = 21) or European (EHB) (N = 38) colonies. Descriptive statistics for the cavity attributes are shown in Table 2 and Figure 2.

| | time occupied | | | turnover | | | AHB versus EHB | |
|---|---|---|---|---|---|---|---|---|
| | rho | Z-value | p-value | rho | Z-value | p-value | Z-value | p-value |
| entrance height (m) | 0.165 | 1.475 | 0.4102 | -0.068 | -0.606 | 0.5443 | -0.945 | 0.3449 |
| number of entrances | 0.072 | 0.642 | 0.5211 | 0.022 | 0.193 | 0.8472 | -1.033 | 0.3015 |
| entrance size* ($cm^2$) | 0.063 | 0.564 | 0.5730 | -0.034 | -0.300 | 0.7644 | -0.239 | 0.8109 |
| entrance orientation | -0.037 | -0.308 | 0.7579 | 0.145 | 1.223 | 0.2213 | -0.199 | 0.8427 |
| dbh (cm) | 0.061 | 0.559 | 0.5763 | -0.085 | -0.774 | 0.4386 | -0.312 | 0.7551 |
| tree height (m) | 0.123 | 1.124 | 0.2611 | -0.183 | -1.667 | 0.0956 | -0.043 | 0.9654 |
| basal area ($m^2$/ha) | -0.200 | -1.825 | 0.680 | -0.051 | -0.467 | 0.6406 | -0.639 | 0.5228 |
| canopy closure (%) | 0.009 | 0.080 | 0.9360 | 0.010 | 0.090 | 0.9285 | -0.983 | 0.3258 |
| ground cover monocot (%) | -0.158 | -1.451 | 0.1467 | -0.114 | -1.042 | 0.2975 | -1.416 | 0.1568 |
| ground cover dicot (%) | 0.190 | 1.737 | 0.0824 | 0.115 | 1.057 | 0.2905 | -1.476 | 0.1401 |
| motte size ($m^2$) | 0.097 | 0.768 | 0.4423 | -0.115 | -0.906 | 0.3649 | -0.197 | 0.8439 |

* based on the area of an ellipse using entrance width and height

aggregated (Table 16, Figure 25). Occupied cavities were aggregated in distribution for all years examined (Table 16, Figures 28-35).

### Spatial and temporal patterns of Africanized and European colonies

The first Africanized honey bee colony in the study area was identified in 1993 (Table 17, Figure 28). At that time, European colonies were aggregated (Table 17, Figure 28). In 1994, three Africanized colonies were found and European colonies were aggregated (Table 17, Figure 29). The distributions of European and Africanized colonies were aggregated in 1995, when 82.7 % of the colonies were European (Table 17, Figure 30). In 1996, there were equal numbers of European and Africanized colonies, both with aggregated distributions (Table 17, Figure 31). By 1997, 62.9 % of the colonies were Africanized, both with random distributions (Table 17, Figure 32). From 1998 through 2000, 73.7, 80.3, and 80.3 % of colonies were Africanized (Table 17). For each of these years, the distribution of Africanized honey bee colonies was aggregated and the distribution of European honey bee colonies was random (Table 17, Figures 33-35).

## DISCUSSION

### Colony density

The densities of up to 12.5 colonies per $km^2$ observed for this study were the highest reported to date for an area including both suitable and unsuitable habitat (Table 18). The live oak and riparian woodland habitats were the only areas providing suitable

Table 16. Spatial and temporal patterns identified by a nearest neighbor analysis of cavities used by feral honey bee colonies on the Welder Wildlife Refuge. The mean nearest neighbor distance (nnd), standard deviation of the nnd, the mean distance expected for a random distribution (rand), the mean distance expected for a dispersed distribution (disp), the nearest neighbor index (nn index), the Z statistic used to test for significance, and the resulting distribution are reported.

| year | sample size | mean nnd | stdev nnd | mean rand | mean disp | nn index | Z | distribution |
|---|---|---|---|---|---|---|---|---|
| 1993 | 25 | 112.46 | 126.09 | 192.45 | 413.58 | 0.5843 | -3.9759 | aggregated |
| 1994 | 62 | 76.14 | 68.75 | 142.55 | 306.34 | 0.5341 | -7.0181 | aggregated |
| 1995 | 81 | 64.60 | 65.34 | 124.71 | 268.02 | 0.518 | -8.2991 | aggregated |
| 1996 | 32 | 120.71 | 112.33 | 177.64 | 381.76 | 0.6795 | -3.4679 | aggregated |
| 1997 | 35 | 133.18 | 142.17 | 182.46 | 392.11 | 0.7299 | -3.0567 | aggregated |
| 1998 | 38 | 117.11 | 128.02 | 174.5 | 375.01 | 0.6711 | -3.8787 | aggregated |
| 1999 | 61 | 83.15 | 94.43 | 141.61 | 304.33 | 0.5871 | -6.1686 | aggregated |
| 2000 | 76 | 82.71 | 86.5 | 127.5 | 274.01 | 0.6487 | -5.8585 | aggregated |
| all cavities | 108 | 62.4 | 57.91 | 108.01 | 232.11 | 0.5778 | -8.394 | aggregated |

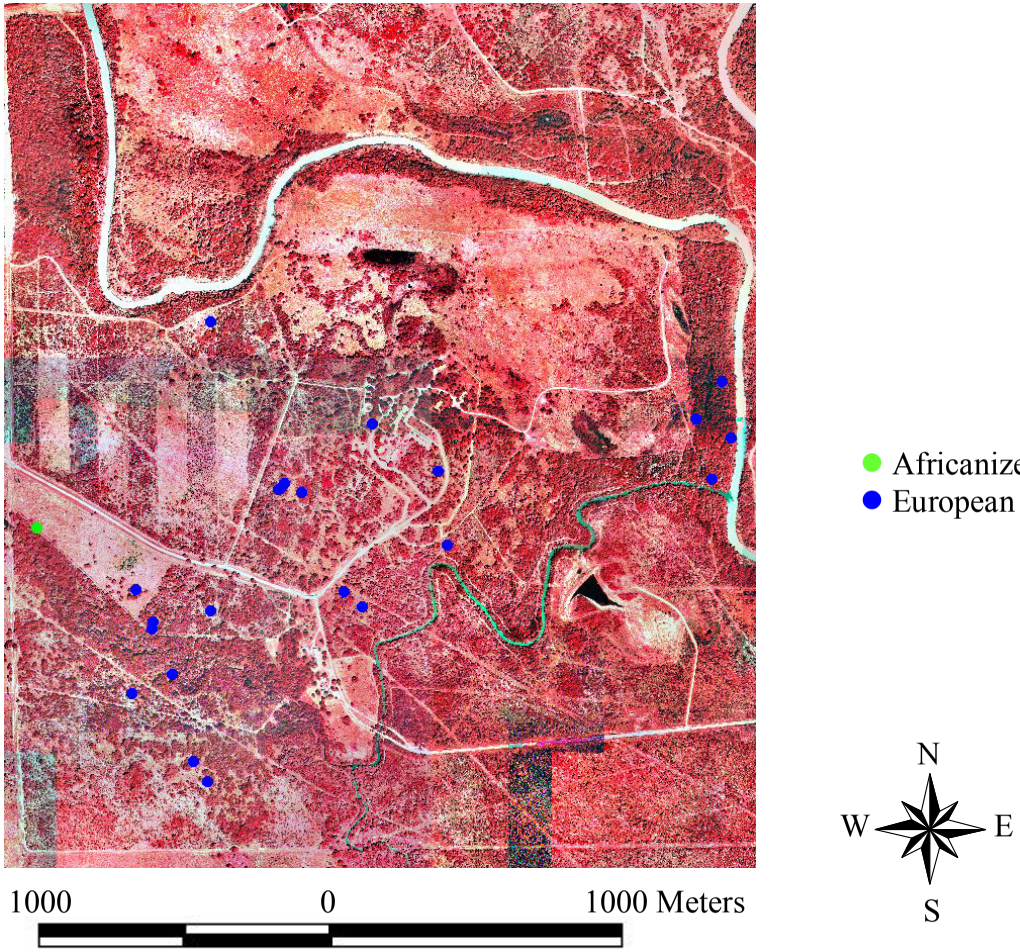



Figure 28. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1993.

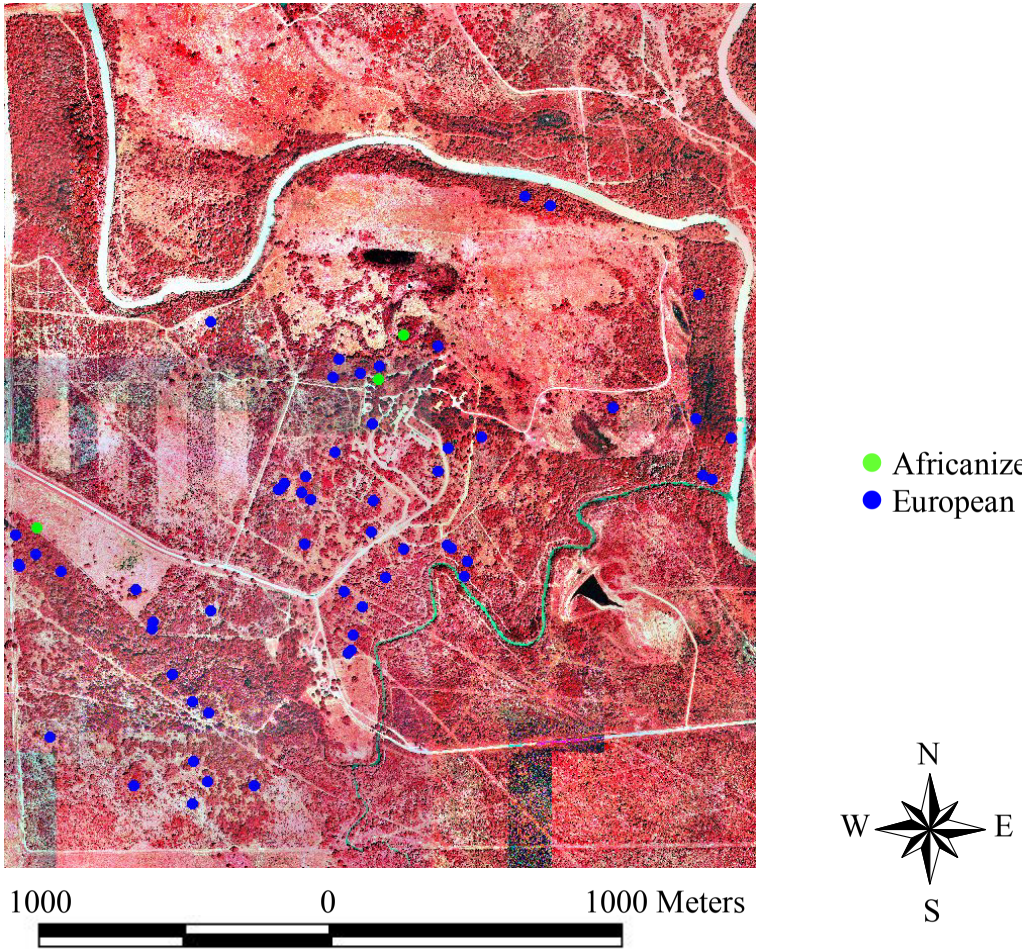



Figure 29. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1994.

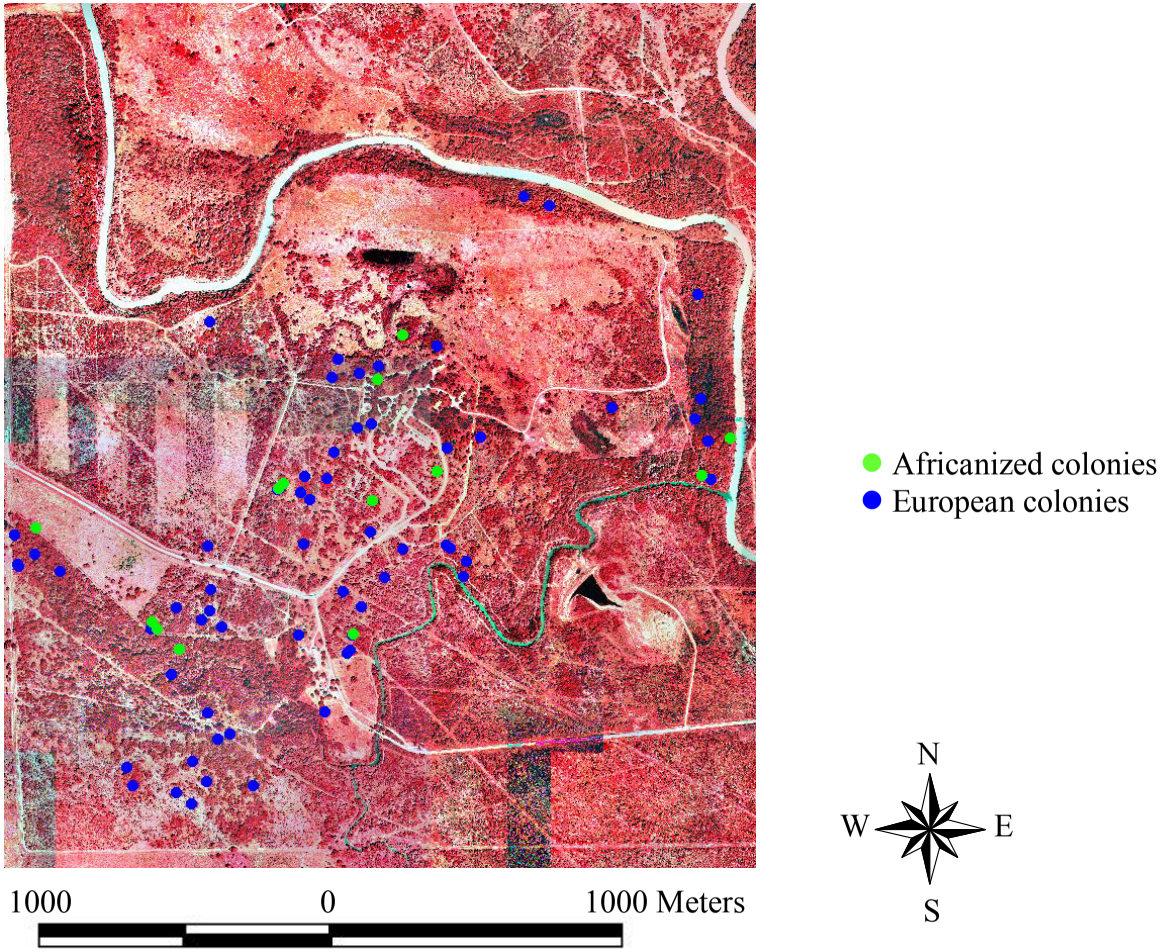



Figure 30. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1995.

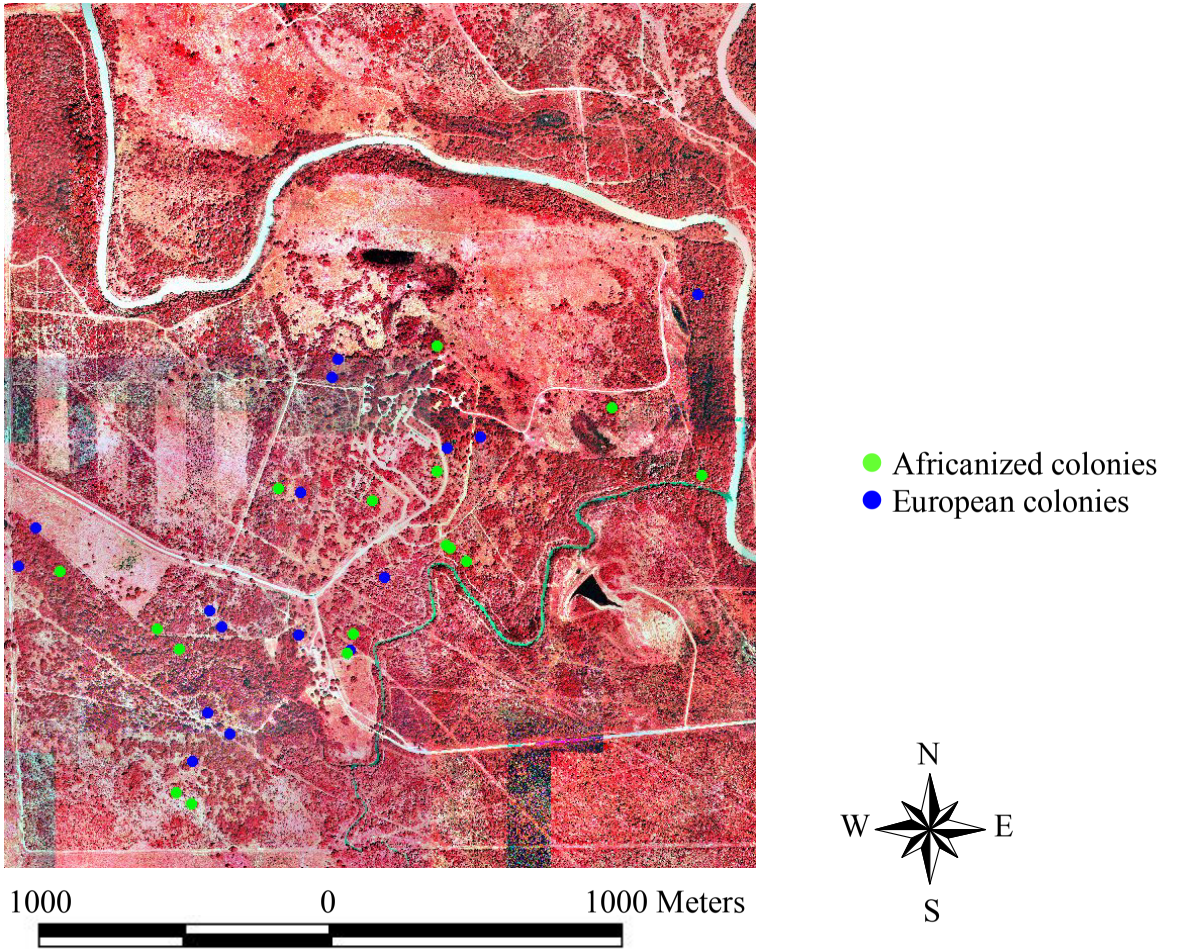



Figure 31. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1996.

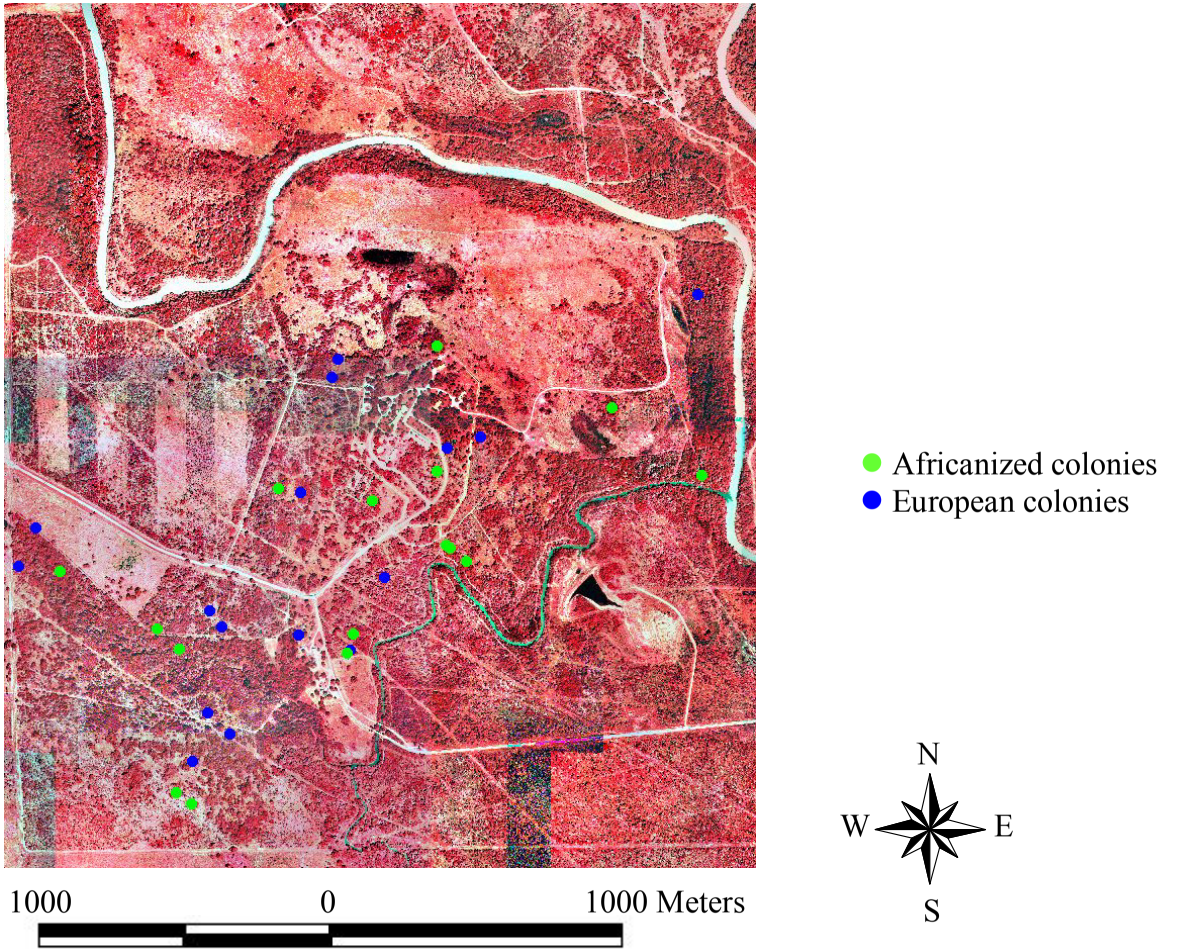



Figure 32. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1997.

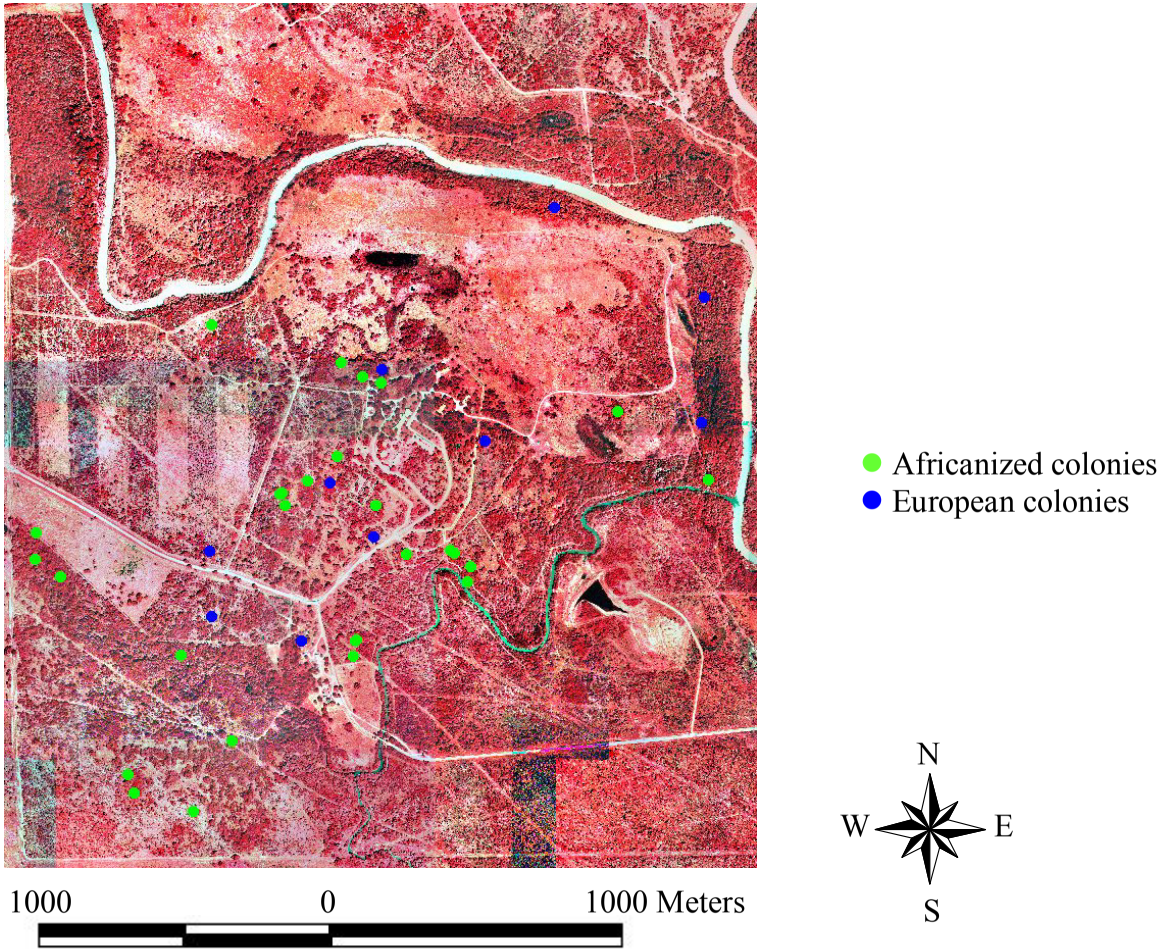



Figure 33. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1998.

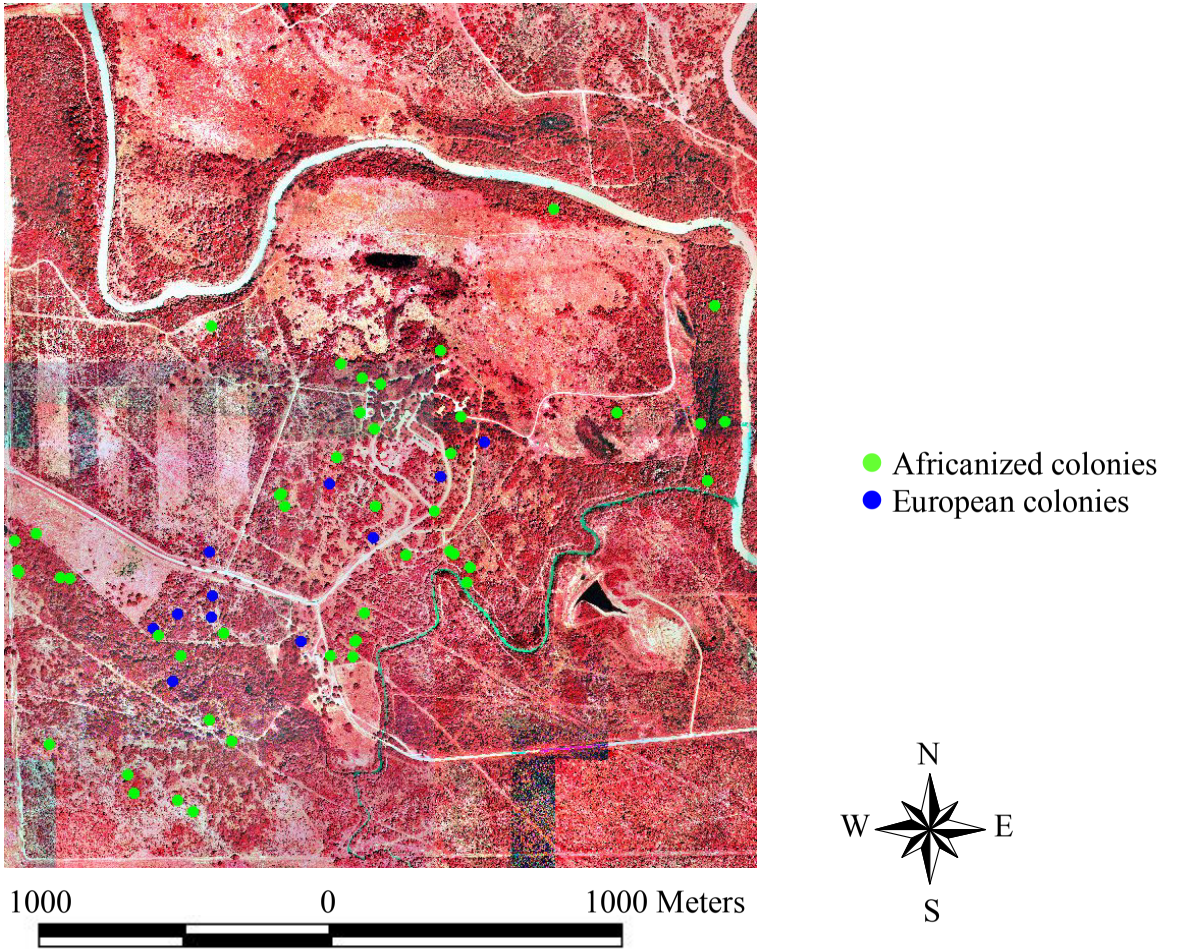



Figure 34. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 1999.

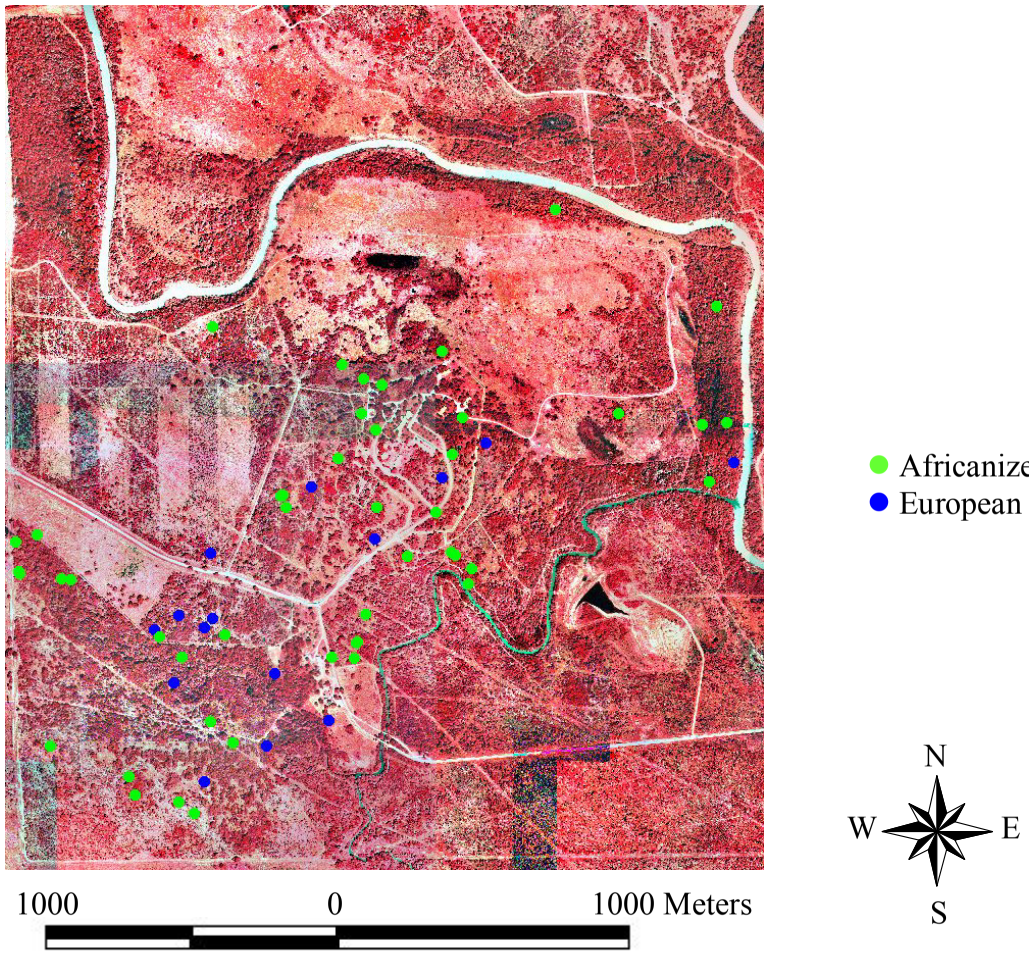



Figure 35. Location of Africanized and European honey bee colonies on the Welder Wildlife Refuge in 2000.

Table 17. Spatial and temporal patterns identified by a nearest neighbor analysis of cavities occupied by European (E) and Africanized (A) colonies on the Welder Wildlife Refuge. The mean nearest neighbor distance (nnd), standard deviation of the nnd, the mean distance expected for a random distribution (rand), the mean distance expected for a dispersed distribution (disp), the nearest neighbor index (nn index), the Z statistic used to test for significance, and the resulting distribution are reported.

| | sample size | mean nnd | stdev nnd | mean rand | mean disp | nn index | Z | distribution |
|---|---|---|---|---|---|---|---|---|
| 1993A | 1 | na | na | na | na | na | na | na |
| 1993E | 24 | 95.97 | 116.92 | 182.47 | 392.13 | 0.526 | -4.4427 | aggregated |
| 1994A | 3 | na | na | na | na | na | na | na |
| 1994E | 59 | 78.28 | 71.69 | 146.13 | 314.04 | 0.5357 | -6.8231 | aggregated |
| 1995A | 14 | 130.24 | 121.42 | 213.86 | 459.6 | 0.609 | -2.7989 | aggregated |
| 1995E | 67 | 77.02 | 68.22 | 135.23 | 290.61 | 0.5696 | -6.7399 | aggregated |
| 1996A | 16 | 171.97 | 142.43 | 230.81 | 496.03 | 0.7451 | -1.9508 | aggregated |
| 1996E | 16 | 187.21 | 190.93 | 239.89 | 515.55 | 0.7804 | -1.6807 | aggregated |
| 1997A | 22 | 165.11 | 171.5 | 201.93 | 433.95 | 0.8177 | -1.636 | random |
| 1997E | 13 | 278.39 | 148.03 | 263.98 | 567.31 | 1.0546 | 0.3767 | random |
| 1998A | 28 | 116.25 | 143.72 | 182.41 | 392.01 | 0.6373 | -3.6716 | aggregated |
| 1998E | 10 | 288.61 | 151.37 | 247.23 | 531.31 | 1.1674 | 1.0127 | random |

Table 17. Continued.

| | sample size | mean nnd | stdev nnd | mean rand | mean disp | nn index | Z | distribution |
|---|---|---|---|---|---|---|---|---|
| 1999A | 49 | 91.44 | 104.49 | 158 | 339.56 | 0.5787 | -5.642 | aggregated |
| 1999E | 12 | 161.59 | 118.24 | 168.23 | 361.54 | 0.9606 | -0.2614 | random |
| 2000A | 61 | 84.42 | 93.87 | 141.61 | 304.33 | 0.5961 | -6.0346 | aggregated |
| 2000E | 15 | 192.57 | 201.44 | 193.03 | 414.84 | 0.9976 | -0.0178 | random |

na = not applicable (sample size too small for analysis)

Table 18. Feral colony densities reported in the literature and this study. Format follows Ratnieks et al. (1991), with the inclusion of more recent data.

| Citation | location | habitat | nest site | area ($km^2$) | density ($km^2$) |
|---|---|---|---|---|---|
| †Galton 1971 ( from Ratnieks et al. 1991) | Russia | temperate forest | hollow trees | 10.4 | 0.96 |
| †Galton 1971 ( from Ratnieks et al. 1991) | Russia | temperate forest | hollow trees | 88 | 0.57 |
| †Galton 1971 ( from Ratnieks et al. 1991) | Russia | temperate forest | hollow trees | 10 | 0.3 |
| †Galton 1971 ( from Ratnieks et al. 1991) | Russia | temperate forest | hollow trees | 72.5 | 0.17 |
| †Kerr 1974 (from Morse et al. 1990) | Brazil | small patches of forest | nr | nr | 2.1 |
| Taber 1979 | Arizona | semi-desert canyon | small rock caves | 3.1 | 2.9-5.1* |
| Visscher and Seeley 1982 | New York | temperate forest | hollow trees | 16.4 | 0.5 |
| Boreham and Roubik 1987 | Panama Canal | various | buildings, hollow trees, open | 50 | 4.7-7.1** |
| Schneider and Blyther 1988 | Okavango River Delta, Botswana | semi-desert | hollow trees, termite nests | 6 | 7.8 |
| Wenner 1989 | Santa Cruz Island | arid island | small rock caves | 230 | 0.25 |
| †unpublished data, F. L. W. Ratnieks (from Morse et al. 1990) | Chiapas, Mexico | banana cultivation | nr | nr | 6.2 |
| Morse et al. 1990 | New York | urban, suburban | buildings, hollow trees | 4.8 | 2.7 |
| Ratnieks et al. 1991 | Chiapas, Mexico (San Roque) | agricultural land | hollow trees, termite nests, open | 2.1 | 6.2 |
| Ratnieks et al. 1991 | Chiapas, Mexico (Huixtla Road) | agricultural land | hollow trees, termite nests, open | ca. 1 | 5 |
| Ratnieks et al. 1991 | Chiapas, Mexico (Rio Florido | agricultural land | hollow trees, termite nests, open | ca. 1 | 9 |
| Oldroyd et al. 1994 | northwest Victoria, Australia | national park | hollow trees | 0.35 | 7.71*** |
| McNally and Schneider 1996 | Okavango River Delta, Botswana | semi-desert | hollow trees, termite nests, open | ca. 50.3 | 1.8-4.2 |
| this study | Texas | coastal prairie | hollow trees | 6.25 | 4.5-12.5 |

nr = not reported

† original not consulted

* over duration of five year study

** only from newly established colonies

*** Reported as 77.1, but as the authors point out, the actual density of colonies in the area is much lower, since the study site comprised only a 100 m wide swath of suitable habitat. Therefore, I converted the reported value into an estimate of the density of feral colonies within a 1 km by 1 km area, assuming feral colonies were only found in the 100 m wide swath of suitable habitat.

cavities, and these habitats comprised 44 % of the study area (Figure 25). Oldroyd et al. (1994) reported a density of 77.1 colonies per $km^2$, but only considered a narrow swath of suitable habitat 100 m wide. When considering a square area (1 km by 1 km), the density is actually 7.71 colonies per $km^2$, with suitable habitat comprising only 10 % of the total area. Data reported in Kerr (1971) were omitted for similar reasons (Ratnieks et al. 1991) because detailed information was not available to convert the data into a comparable format. Based on these considerations, the previously reported highest densities were 7.8 (Schneider and Blyther 1988), 7.7 (Oldroyd et al. 1994), and 7.1 (Boreham and Roubik 1987) colonies per $km^2$. Therefore, the highest density reported for this study is much higher than previously reported densities.

In general, the study area appears to be highly suitable for feral honey bee colonies. Cavity density is high in certain areas, and pollen and nectar sources are abundant throughout most of the year (unpublished data, K. A. Baum, W. L. Rubink, and R. N. Coulson). Conservative estimates of annual pollen and nectar production for plants in the study area based on abundance and growth form suggest that 1895 feral colonies could be supported by pollen sources and 244 feral colonies could be supported by nectar sources within the study area, based on the annual resource requirements of a typical feral colony (unpublished data, K. A. Baum, W. L. Rubink, and R. N. Coulson). These estimates increase to 3161 feral colonies for pollen sources and 407 feral colonies for nectar sources when the area is expanded beyond the study site boundaries to include the entire potential foraging range of the feral colonies, based on the spatial locations of cavities and a foraging radius of 800 m (unpublished data, K. A. Baum, W. L. Rubink,

and R. N. Coulson). The densities reported in this study are high for natural areas (Table 18). However, even higher densities may occur in urban landscapes where honey bee colonies nest in human made structures and landscaping practices provide pollen and nectar during natural periods of resource dearth (unpublished data, S. C. Thoenes).

Cavity attributes

Compared to other studies (Table 19), cavities used by feral colonies occurred in a relatively few number of tree genera. However, Oldroyd et al. (1994) reported the use of only *Eucalyptus*. *Quercus*, the most common tree used by feral colonies in this study, was also frequently used in other areas. Feral colonies usually occupied cavities located in living trees, although Seeley and Morse (1976) reported 25 % of colonies using cavities in dead trees. Most occupied cavities had a single entrance, which also was reported in other studies. Mean tree dbh was typically over 70 cm. Entrance height varied, but may be more of a function of available options than a preference for the reported heights. Entrance areas recorded in this study were smaller than those reported by others (Table 19). Entrance orientation was commonly to the northwest or southeast (Figure 24).

In most cases, the observed cavity attributes were similar to those reported from other areas (Table 19). However, cavity constraints on feral colonies vary depending on geographic location. For example, tropically adapted Africanized honey bees typically have smaller colony sizes and store less honey than temperately adapted European honey bees (Winston et al. 1981). Therefore, Africanized colonies often utilize smaller cavities

Table 19. Cavity attributes compared between this study and similar data reported in the literature. Format follows Gambino et al. (1990), with corrections and some modifications. Data were often based on less than the sample size (total number of nests).

| | | this study | Avitabile et al. 1978 | Seeley and Morse 1976 | Gambino et al. 1990 | Oldroyd et al. 1994 |
|---|---|---|---|---|---|---|
| | location | Texas | Connecticut | New York | California | Australia |
| | number nests | 108 | 108 | 39 | 94 | 51 |
| | number host genera | 5 | 16 | 12 | 14 | 1 |
| | most common | *Quercus* (84%) | *Quercus* (20%) | *Acer* (33%) | *Quercus* (52%) | *Eucalyptus* (100%) |
| | second most common | *Celtis* (6%) | *Juglans* (13%) | *Quercus* (25%) | *Eucalyptus* (12%) | na |
| | live trees | 94% | 94% | 75% | 94% | 88.5% |
| | single entrance | 88% | 90% | 79% | 94% | nr |
| tree dbh (cm) | mode | 75-100 | 90-100 | nr | nr | 25-50, 75-100 |
| | mean | 74.96 | nr | 90 | 85.59 | 87.4 |
| | range | 30-185 | nr | 30-180 | 38-152 | >0-≤150 |

Table 19. Continued.

| | | this study | Avitabile et al. 1978 | Seeley and Morse 1976 | Gambino et al. 1990 | Oldroyd et al. 1994 |
|---|---|---|---|---|---|---|
| entrance height (m) | mode class | 2.5-3 | 2-3 | 0-1 | 0-0.5 | 8-9 |
| | median | 2.2 | 3-4 | 1-2 | 1.53 | 7-8 |
| | mean | 2.5 | 4.15 | nr | 2.28 | 7.5, 5.2, 1.6 |
| entrance area ($cm^2$) | mode class | 10-20* | 35-40 | 10-20 | 20-40 | 0-100 |
| | median | 15.7* | 60-65 | 20-30 | 46 | 100-200 |
| | mean | 42.10 | 131 | nr | 110.3 | nr |
| | entrance orientation | nw, se | 225 | random | random | random |

nr = not reported

* based on the area of an ellipse using entrance width and height

than European colonies (Seeley and Morse 1976, Seeley 1977). These differences highlight selection pressures faced by feral honey bees in different geographic locations (Winston et al. 1983).

Preferences for different nest site characteristics have been proposed for Africanized and European honey bees. Schmidt and Hurley (1995) reported that Africanized honey bees showed no preference for cavity sizes ranging from 13.5 to 30 l, while European honey bees preferred larger cavity sizes. However, no differences were found between cavities occupied by Africanized or European colonies in this study, so the structural and environmental attributes of cavities do not differ between those occupied only by Africanized or only by European colonies (Table 15). Cavity volume could not be measured, so perhaps differences do exist in volume between cavities used by Africanized and European colonies in the study area. To date, selection for volume and shape (Schmidt and Thoenes 1992) are the only nest site characteristics that have been compared between European and Africanized colonies.

With the exception of Taber (1979), no other published studies have examined cavity occupancy through time. The time occupied and turnover indices provide different information about the quality of a cavity. Cavities occupied a majority of the time, but with high turnover rates, may not be as suitable for feral colonies as cavities occupied for long periods of time with little or no turnover. Therefore, together these indices provide an estimate of overall cavity quality. However, none of the measured cavity attributes were correlated with the time occupied and turnover indices, so cavities do not vary in their suitability for honey bees based on the measured structural and

environmental attributes (Table 15). Other studies have documented preferences for certain nest site characteristics. For example, colonies selected nest sites 5 m and 3 m off the ground over nest sites 1 m off the ground (Seeley and Morse 1978, Schmidt and Thoenes 1987). However, these preferences have seldom been directly related to corresponding data on colony survival, growth, and reproduction and are not comparable to this study in terms of the time occupied and turnover indices. It was not possible to measure cavity volume and volume is perhaps the most important cavity attribute, or at least the best documented in terms of honey bee preferences.

Spatial and temporal patterns

The distribution of feral colonies was aggregated throughout the time period of this study, so spatial patterns do exist in cavity use by the feral colonies (Table 16). However, few studies have reported aggregations of *A. mellifera* (Oldroyd et al. 1995, McNally and Schneider 1996). The lack of reported aggregations by *A. mellifera* suggests that swarms tend to disperse, suitable nest sites are not common, or no one has conducted surveys for feral colonies (Oldroyd et al. 1995).

Some studies have reported swarms selecting nearby nest sites (Jaycox and Parise 1980, 1981), but others have reported swarms selecting more distant sites (Seeley and Morse 1977). These ambiguous results probably represent genetic differences between the colonies studied and/or local patterns of resource availability (Winston 1987). Colony aggregations may also result from the attraction of swarms to existing colonies not near the parent colony (Oldroyd et al. 1995). These different scenarios can

be evaluated by examining the relatedness of colonies in aggregations. Related colonies would support the short dispersal distance scenario and unrelated colonies would support the attraction scenario. Oldroyd et al. (1995) found some completely unrelated colonies in aggregations in Wyperfeld National Park, northwest Victoria, Australia, rejecting the explanation of short dispersal distances. The familial relationships among colonies have not been examined on the Welder Wildlife Refuge. However, four different mitotypes have been identified (unpublished data, M. A. Pinto, W. L. Rubink, J. S. Johnston, and R. N. Coulson). The number of colonies with different mitotypes through time does not appear to support the short dispersal distance (related colonies) scenario, although low colony or swarm survival could conceal this pattern.

Jaycox and Parise (1980, 1981) and Seeley and Morse (1977) suggested that swarms select nearby cavities when cavity availability is high. Therefore, colony aggregations would be expected when cavities are abundant. Oldroyd et al. (1994) estimated up to 11000 hollows per $km^2$ within the same study area used by Oldroyd et al. (1995), suggesting an abundance of cavities. They also reported that nectar and pollen sources are abundant. However, the area surveyed only formed a 100 m wide swath of suitable habitat (Oldroyd et al. 1995), so cavities may be uncommon at a broader spatial scale (larger extent). The same conclusion could be drawn for the suitable habitat on the Welder Wildlife Refuge. Although suitable habitat is abundant within the refuge and several adjoining counties, live oak mottes, the main cavity source for feral honey bee colonies, are not abundant at a larger scale (Figure 36). Based on vegetation communities defined by McMahan et al. (1984), cavities probably are available in the

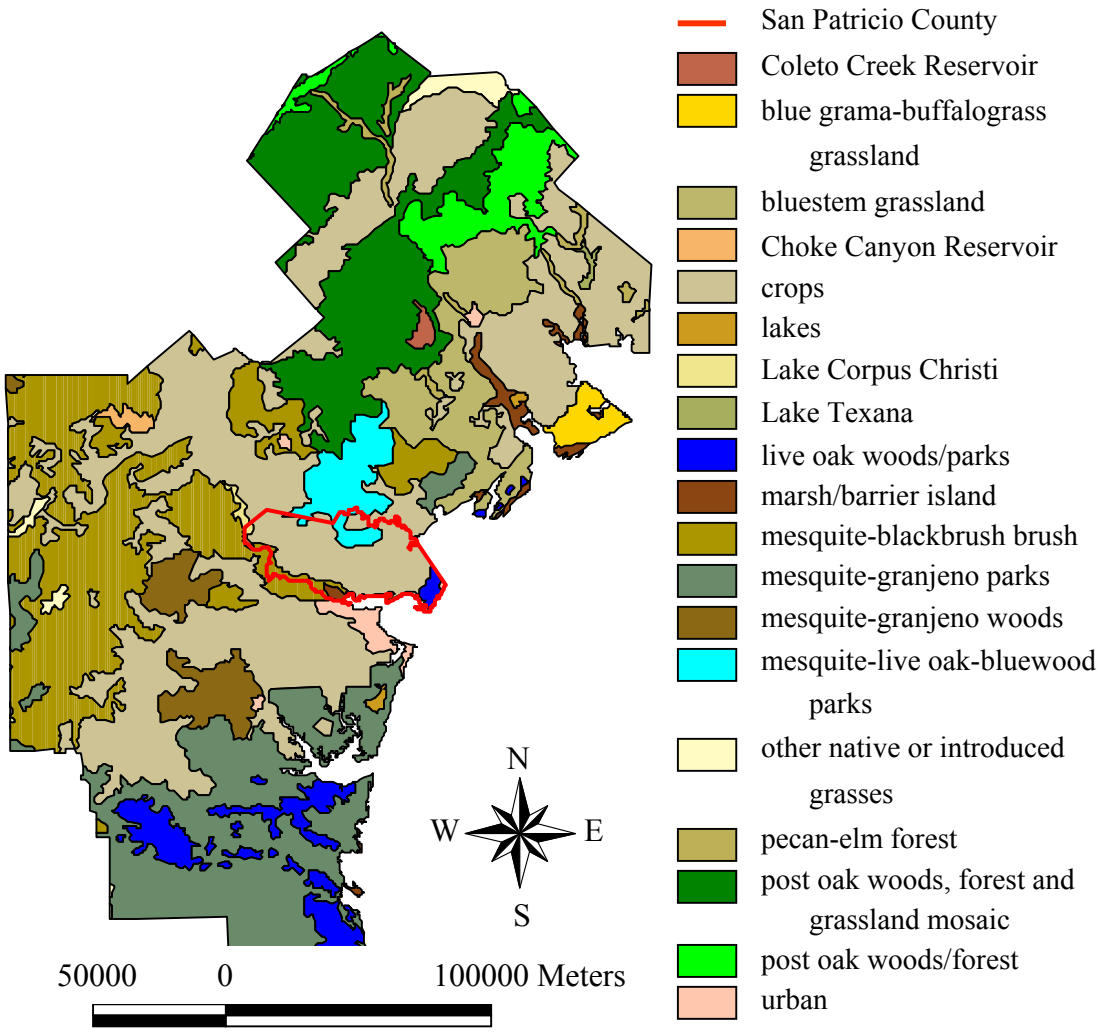



Figure 36. Vegetation classification for the Texas coastal bend from McMahan et al. (1984). The Welder Wildlife Refuge is located in San Patricio County.

Texas coastal bend (including the counties of Gonzales, Lavaca, Dewitt, Victoria, Jackson, Goliad, Calhoun, McMullen, Live Oak, Bee, Refugio, Aransas, San Patricio, Duval, Jim Wells, Nueces, Kleberg, Brooks, and Kenedy) in the mesquite-live oak-bluewood parks, live oak woods/parks, post oak woods, forest and grassland mosaic, and post oak woods/forest. These habitats comprise 22 % of the area, while the highly suitable habitat of mesquite-live oak-bluewood parks found on the Welder Wildlife Refuge makes up only 2 % of the Texas coastal bend region (Figure 36). Thus, the distribution of cavity sources is patchy and potentially rare at a broader spatial scale (large extent).

In addition to dispersal behavior and resource distributions, other proposed hypotheses to explain colony aggregations include predator defenses and mating efficiency (Seeley et al. 1982, Oldroyd et al. 1995). There is controversy over whether aggregations would serve to decrease or increase the probability of predation (Seeley et al. 1982, Oldroyd et al. 1995). However, aggregations may increase predator detection, since colonies may become alerted when a nearby colony is disturbed (Seeley et al. 1982). Possible predators on honey bee colonies that are present on the Welder Wildlife Refuge include skunks, birds, opossums, shrews, armadillos, and invertebrates, such as wasps, ants, and moths (Winston 1987). However, these animals probably have a minimal impact on the feral colonies, since most are located in tree cavities several meters off the ground with relatively small entrances. Therefore, the observed aggregated pattern probably does not result from predator defenses.

Lastly, aggregations may increase mating efficiency by decreasing the distance to drone congregation areas. In the case of unrelated aggregations, the probability of mating with brothers would also be decreased (Oldroyd et al. 1995). Mating with brothers results in diploid males and reduces brood viability (Page 1980). Therefore, mechanisms that decrease the probability of mating with brothers should be selected for, such as multiple matings and unrelated aggregations.

Spatial and temporal patterns of Africanized and European colonies

Africanized and European colonies were both aggregated during 1995 and 1996, and both randomly distributed during 1997 (Table 17). Therefore, differences existed in the spatial distribution of Africanized and European colonies during these years. However, from 1998 through 2000, Africanized colonies were aggregated and European colonies were randomly distributed (Table 17). Therefore, spatial patterns do differ between Africanized and European colonies, and these patterns do vary through time.

The spatial and temporal distribution of European and Africanized colonies represents the invasion process as Africanized honey bees arrive and become established in an area with an existing feral population of European honey bees. After the initial two years (1993 and 1994) when sample sizes of Africanized colonies were too small to evaluate spatially, Africanized honey bees were aggregated, with the exception of 1997. The 1997 sampling year appears to be a transition period, with the random distribution of Africanized and European colonies. After that time, European colonies remained randomly distributed, while Africanized colonies were aggregated. Therefore, the

invasion of Africanized honey bees appears to have fragmented the existing European population, corresponding to a decrease in the overall number of European colonies in the study area.

Mitochondrial DNA provides a historical perspective on the invasion of Africanized honey bees, highlighting what happens to the existing and invading colonies and their subsequent offspring. However, mitochondrial DNA only represents the maternal side of the Africanization process. Preliminary conclusions based on nuclear DNA indicate that the Africanized and European colonies on the Welder Wildlife Refuge were separate populations until 1997, when they genetically became the same population (unpublished data, M. A. Pinto, W. L. Rubink, J. S. Johnston, and R. N. Coulson). Therefore, the patterns observed from 1997 through 2000 represent only a subset of the feral population and the resulting patterns may be an artifact of subdividing a single population.

## Conclusions

The colony densities of up to 12.5 colonies per $km^2$ observed in the study area were the highest reported in the literature for an area including both suitable and unsuitable habitat. The measured cavity attributes were similar to those reported from other areas. The time occupied and turnover indices provided useful information about cavity quality. However, none of the measured cavity attributes were correlated with the time occupied and turnover indices. Therefore, cavities appeared not to vary in their

suitability for honey bees based on the measured structural and environmental attributes, but probably varied in quality based on unmeasured cavity characteristics.

Spatial patterns existed in cavity use by the feral colonies, with the colonies showing an aggregated pattern of distribution throughout the time period of this study. Colony aggregations probably resulted from the distribution of resources, especially cavities, although none of the proposed explanations for the formation of colony aggregations could be rejected. The spatial and temporal distribution of European and Africanized colonies represented the pattern of the invasion of Africanized honey bees. Two years after the arrival of Africanized bees, Africanized and European colonies were aggregated. 1997 appeared to be a transition period, with the random distribution of Africanized and European colonies. After that time, European colonies remained randomly distributed, while Africanized colonies were aggregated. Therefore, the invasion of Africanized honey bees appeared to fragment the existing European population, corresponding to a decrease in the overall number of European colonies in the study area.

# CHAPTER IV

# RESOURCE USE – POLLEN

## INTRODUCTION

Pollen is an important protein source for honey bee colonies and is required for brood and young worker development (Maurizio 1950, Haydak 1970). Pollen also provides lipids, carbohydrates, vitamins, and minerals. Honey bees meet their pollen needs by collecting a wide variety of pollen types, including pollen from most angiosperm groups, as well as gymnosperms, ferns, and even non-nutritional particulate matter, such as coal dust and sawdust (Schmalzel 1980, Buchmann et al. 1992). This diet breadth is important for colony survival because honey bee colonies are perennial and active throughout much of the year.

The collection of pollen by honey bees also provides valuable pollination services for many plants, including economically valuable crops, ornamentals, and native species. Traps are often used to collect pollen loads for identification from colonies located in human made hives (Synge 1947, Poulsen 1973, Adams et al. 1978, Severson and Parry 1981, O'Neal and Waller 1984, Pearson and Braiden 1990, Coffey and Breen 1997, Wilms and Wiechers 1997, Nagamitsu and Inoue 1999). In most cases, pollen traps are used for extended periods of time. However, this may alter foraging behavior by decreasing pollen flow into the colony, causing the colony to collect larger quantities of pollen and potentially utilize different pollen sources. Other studies have recorded floral visitation by honey bees and other bees (de Menezes Pedro and de

Camargo 1991). However, recording floral visitations does not provide an accurate measure of the cumulative foraging effort of a colony. No studies have documented pollen use by feral colonies under unmanipulated conditions in natural hive sites. Therefore, I documented pollen use by feral colonies in tree cavities in a coastal prairie landscape, with the goal of examining the foraging ecology of feral colonies under natural conditions. Specific objectives included evaluating overlap in pollen use between pairs of colonies during different sampling periods and examining the influence of the spatial locations of the colonies on overlap in pollen use.

## METHODS

The study site was located on the Welder Wildlife Refuge in San Patricio County, Texas. Feral colonies were found in the western one-quarter of the refuge in live oak mottes and riparian woodland habitat. Over a twelve-year period, 109 cavities were identified that contained a feral colony at one point in time (Figure 37). During the time period of this study, 56 to 64 of those cavities were occupied.

Dead bee traps modified to hold removable pollen screens were placed on six feral colonies located in tree cavities using ratcheting straps and polyurethane foam to seal other potential entrances (Figure 38). Traps were also placed on three feral-origin colonies located in Langstroth hives, and one feral-origin colony in a Kenya top-bar hive, none of which received any management. Colonies were selected based on their suitability for the use of a dead bee trap (cavity height, entrance size, and entrance shape) and their distance from other colonies with pollen collection traps (Figure 37).

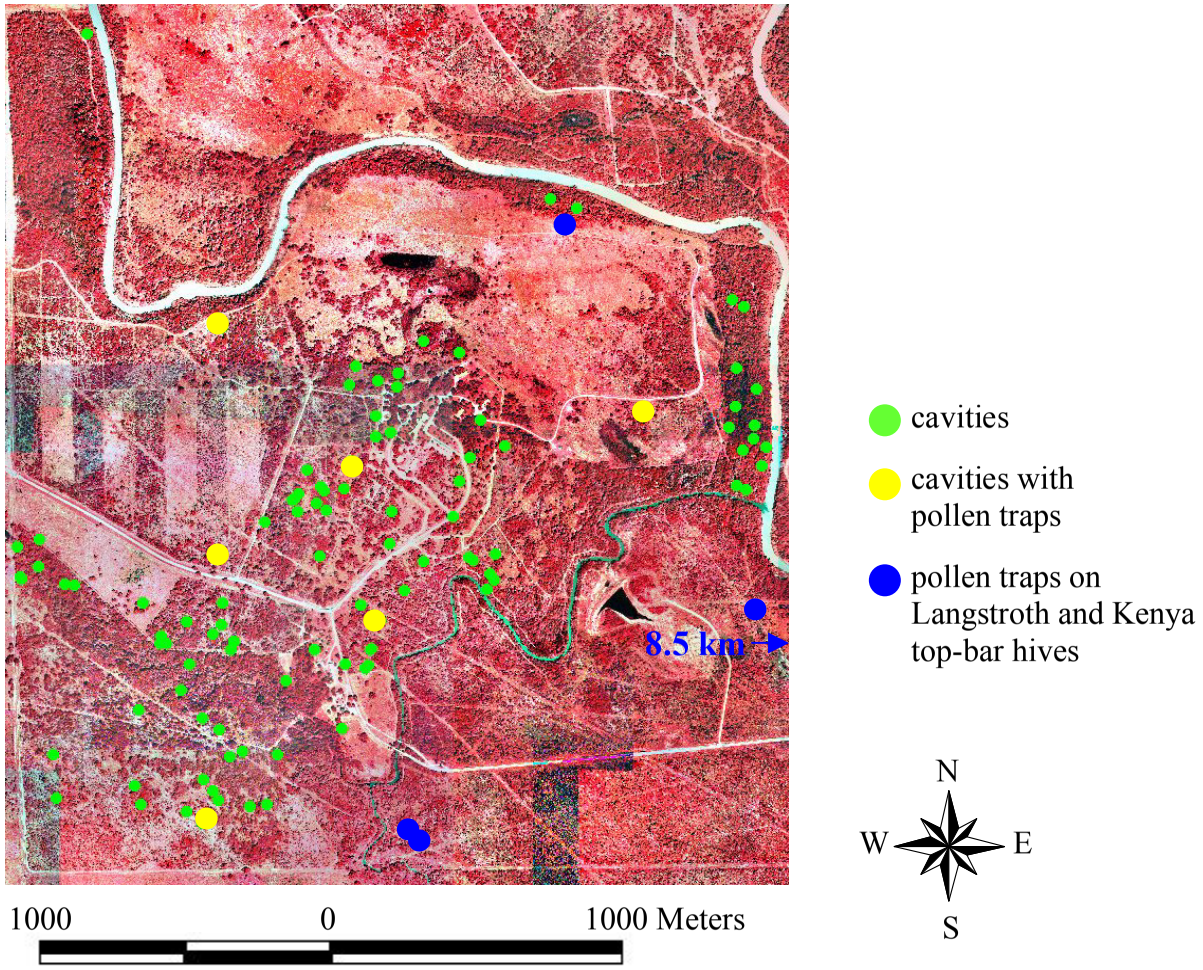



Figure 37. Cavity and pollen trap locations.

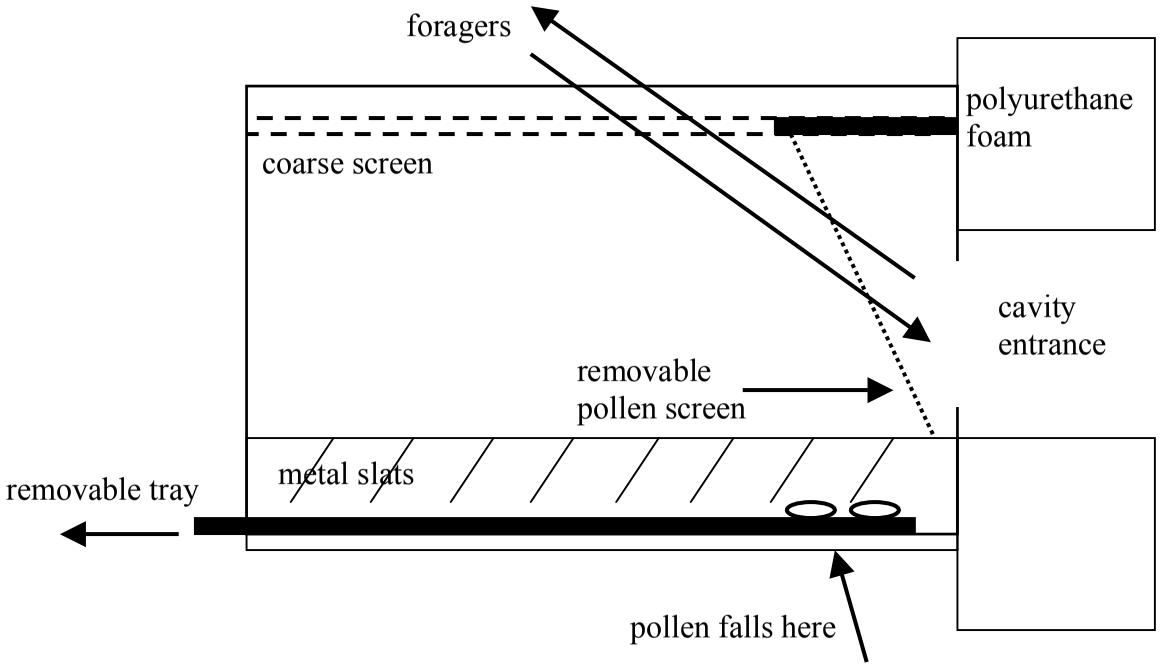



Figure 38. Dead bee trap modified to hold a removable pollen screen. Polyurethane foam around the cavity entrance forced returning foragers to enter through the trap. The corbicular pollen loads of returning foragers were knocked off as the bees entered the hive through the pollen screen. Pollen fell through the metal slats onto a removable tray, which prevented the bees from collecting the pollen pellets.

The colonies located in the Langstroth hives and the Kenya top-bar hive were located in areas where occupied cavities were not available during the study period or not suitable for a pollen trap.

Pollen screens consisted of three layers of wire mesh with 0.45 cm openings between wires. Each layer was offset and separated from the other layers by about 0.45 cm. Honey bees passed through the screens to enter the hive, knocking off their corbicular pollen loads. Pollen was collected from each colony during an approximately three-hour sampling period once every three weeks from July 2000 through July 2001. However, the length of the sampling period was extended under cool or adverse weather conditions in order to obtain a sample representative of a colony's foraging effort.

Pollen samples were sent to the Palynology Laboratory[3] at Texas A&M University for processing and identification. Each sample was processed using standard acetolysis procedures. First, the sample was placed in a test tube and dissolved in glacial acetic acid, then centrifuged at 100 rpm for three minutes, decanted, and vortexed. Five ml of the acetoloysis solution (nine parts acetic anhydride and one part sulfuric acid) were added to each test tube. The test tubes were placed in a heating block at 90° Celsius for eight minutes. Next glacial acetic acid was added to the acetolysis mixture and the samples were centrifuged for one minute. The solution was decanted and vortexed and the procedure repeated. The mixture was then washed with distilled water (centrifuged, decanted, and vortexed) until the decanted liquid was no longer dark in

[3] Palynology Laboratory, Texas A&M University, College Station, TX 77843-4352, Director, Dr. Vaughn M. Bryant, Jr.

color and became relatively clear. Then the mixture was rinsed with 95 % alcohol and subsampled until one ml remained. The samples were rinsed into one dram glass vials, centrifuged, and decanted. Four drops of glycerin were added to each sample and the vials were placed on a heating block overnight to evaporate any remaining alcohol.

Slides were made for each sample by thoroughly mixing the sample, placing several drops on a slide, spreading the drops over an area the size of the cover slip to assure adequate dispersion, and adding a cover slip. Pollen types were identified to the family or genus level using light microscopy. The level of identification depended on diagnostic characteristics of the pollen types in a given group of plants. A minimum of 200 pollen grains distributed among at least three transects was counted per sample in order to place the pollen types into frequency classes (Louveaux et al. 1978).

I compared the percent overlap of pollen types between each pair of colonies using $100(1 - 0.5\sum_i |Px,i - Py,i|)$, where $Px,i$ and $Py,i$ are the frequencies for colonies x and y of pollen type i (Schoener 1970). A Wilcoxon signed rank test was used to compare overlap between sampling periods and a Spearman rank correlation coefficient to test for correlation between overlap and distance between colonies.

To evaluate further spatial patterns of pollen use, the habitat types with predominant (> 45 % of a sample from any colony during any sampling period) and cumulatively important pollen types (> 8 % of the combined foraging effort of all sampled colonies for a given sampling period) were identified. The abundance of each

plant in each habitat type was obtained from the Welder plant list[4] (unpublished data, Welder Wildlife Foundation). A landscape classification of the study area based on vegetation communities was used to examine spatially the distribution of collected pollen types during each sampling period (Figure 39) (unpublished data, K. A. Baum, W. L. Rubink, and R. N. Coulson).

I made rough estimates of the nutritional contribution of each pollen type by placing each into general size categories and using protein content values reported in Roulston et al. (2000). General size categories included 0 – 2, > 2 – 14, > 14 – 50, > 50 – 100, and > 100 $\mu m^3$. Protein content values were generalized to the genus or family level in order to correspond to the level of identification, since Roulston et al. (2000) found that protein content was highly conserved within plant genera, families, and divisions. I used estimates of nutritional contribution based on general size categories to identify pollen types overestimated or underestimated by only examining frequencies.

I also evaluated pollen collection patterns by examining the contribution of entomophilous versus anemophilous pollen types, herbaceous versus woody pollen sources, and nectariferous versus non-nectariferous pollen sources. These data provide generalized information about pollen collection patterns that can be compared to other areas with different plant communities.

[4] The Welder plant list contains commonly encountered plants on the Welder Wildlife Refuge based on cover data from point frame transects surveyed from 1975 through 1984 by D. Lynn Drawe.

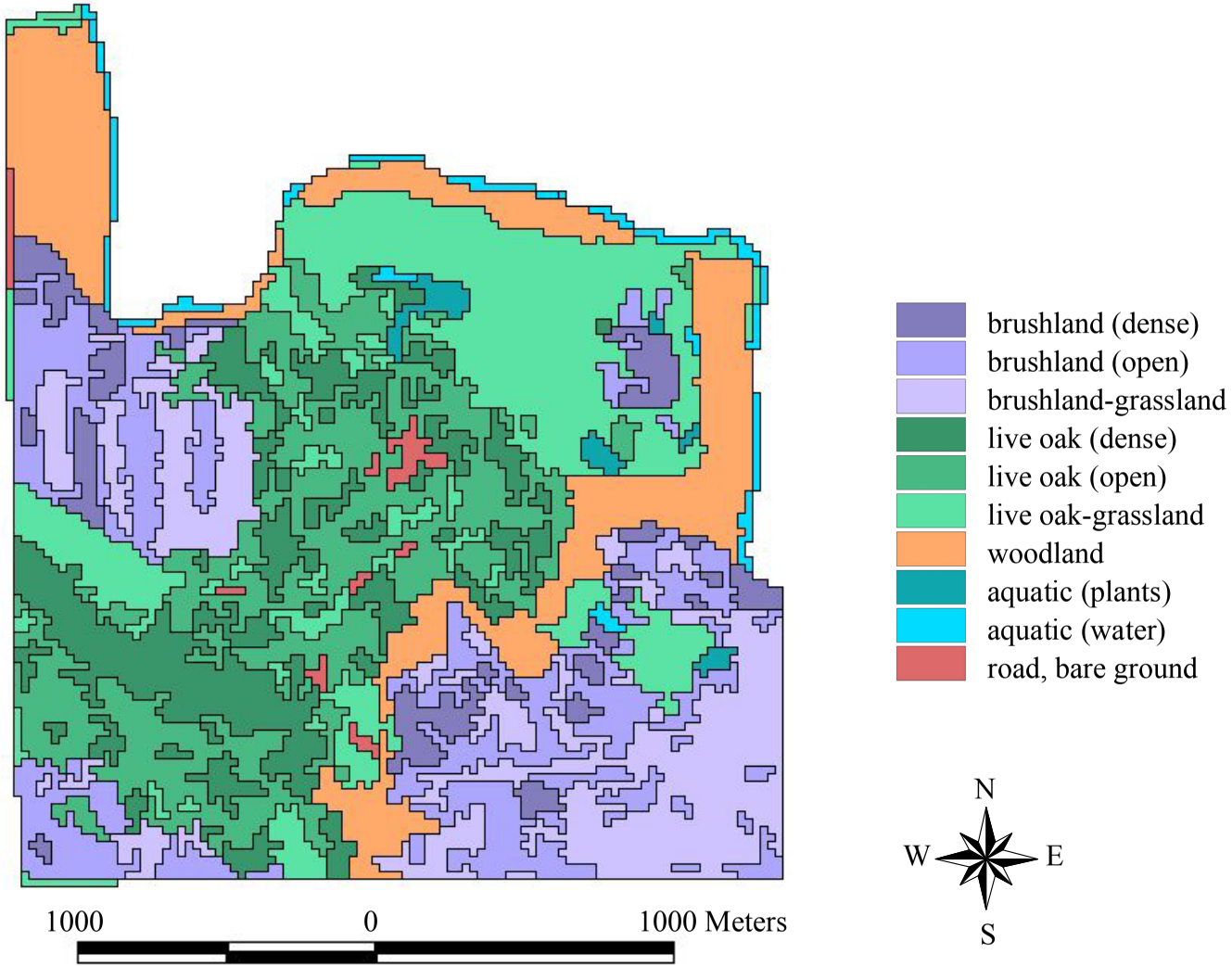



Figure 39. A landscape classification based on vegetation communities showing the spatial distribution of habitats in the study area.

RESULTS

A total of 95 pollen types were collected by the feral colonies throughout the year, including 43 families, 66 genera, and 29 unknown taxa (Table 20, Figure 40). The genera category included pollen types identified to the genus level, as well as those implied to be different genera at the family level. For example, pollen types identified as *Salvia* (family Lamiaceae) and Lamiaceae were different pollen types, because those identified as Lamiaceae were not *Salvia* and belonged to a different genus. Unknown taxa typically comprised a very small portion of a sample, often only a few grains. Only two unknown taxa comprised greater than 25 % of a sample, and those occurred during the February and November sampling periods. The samples included 19 predominant (> 45 % of a sample from any colony during any sampling period) and cumulatively important pollen types (> 8 % of the combined foraging effort of all sampled colonies for a given sampling period) (Table 21).

Four pollen collection periods were identified from March through mid May, late May through early August, mid August through mid December, and February, based on the pollen types collected throughout the year (Table 21). Late December through January was a period when the colonies collected very little pollen. The spring collection period was dominated by *Rhus* I and *Rhus* II. Additional pollen types included Anacardaceae, high spine Asteraceae, low spine Asteraceae, Lamiaceae, *Lythrum*, *Argemone*, and *Salix*. The summer collection period consisted mainly of *Prosopis*, with additional input from Apiaceae, Arecaceae, *Mimosa*, and Poaceae. The fall collection period contained low spine Asteraceae, *Croton*, and *Celtis*, with some

Table 20. Identified pollen types by family.

| | |
|---|---|
| Acanthaceae *Ruellia* | Hydrophyllaceae *Phacelia* |
| Aceraceae *Acer* | Juglandaceae *Carya* |
| Alismataceae *Alisma* | Lamiaceae |
| Alismataceae *Echinodorus* | Lamiaceae *Salvia* |
| Alismataceae *Sagittaria* | Liliaceae |
| Amaryllidaceae *Nothoscordum* | Liliaceae *Schoenocaulon drummondii* |
| Anacardiaceae | Lythraceae *Lythrum* |
| Anacardaceae *Rhus* I | Magnoliaceae *Liriodendron* |
| Anacardaceae *Rhus* II | Malvaceae |
| Apiaceae | Nyctaginaceae |
| Arecaceae | Nymphaceae *Nelumbo* |
| Asteraceae *Ambrosia* | Oleaceae *Fraxinus* |
| Asteraceae *Artemesia* | Onagraceae *Oenothera* |
| Asteraceae (low spine)* | Papaveraceae *Argemone* |
| Asteraceae (high spine)** | Plantaginaceae *Plantago* |
| Asteraceae Liguliflorae† | Poaceae |
| Berberidaceae *Berberis* | Poaceae *Zea mays* |
| Brassicaceae | Polygonaceae *Polygonum* |
| Chenopodiaceae Cheno-am†† | Portulaceae *Portulaca* |
| Cyperaceae *Carex* | Ranunculaceae |
| Cyrillaceae *Cyrilla* | Rhamnaceae |
| Euphorbiaceae *Cnidoscolus* | Rosaceae |
| Euphorbiaceae *Croton* | Rosaceae *Prunus* |
| Fabaceae | Saliaceae *Salix* |
| Fabaceae *Acacia* | Scrophulariaceae |
| Fabaceae *Dalea* | Scrophulariaceae *Pedicularis* |
| Fabaceae *Leucaena* | Solanaceae |

Table 20. Continued.

| | |
|---|---|
| Fabaceae *Mimosa* | Tiliaceae *Tilia* |
| Fabaceae *Mimosa strigillosa* | Ulmaceae *Celtis* |
| Fabaceae *Prosopis* | Ulmaceae *Ulmus* |
| Fabaceae *Trifolium* | Verbenaceae *Phyla* |
| Fagaceae *Quercus* | Verbenaceae *Verbena* |
| Fumariaceae *Corydalis* | Vitaceae *Vitis* |

* Asteraceae (low spine) represents the ragweed group of anemophilous species.

** Asteraceae (high spine) represents the sunflower group of entomophilous species.

† Asteraceae Liguliflorae represents the dandelion group of entomophilous species.

†† Cheno-am represents the Chenopodiaceae and *Amaranthus* in the Amaranthaceae.


Figure 40. Light micrographs of pollen types collected by honey bees.

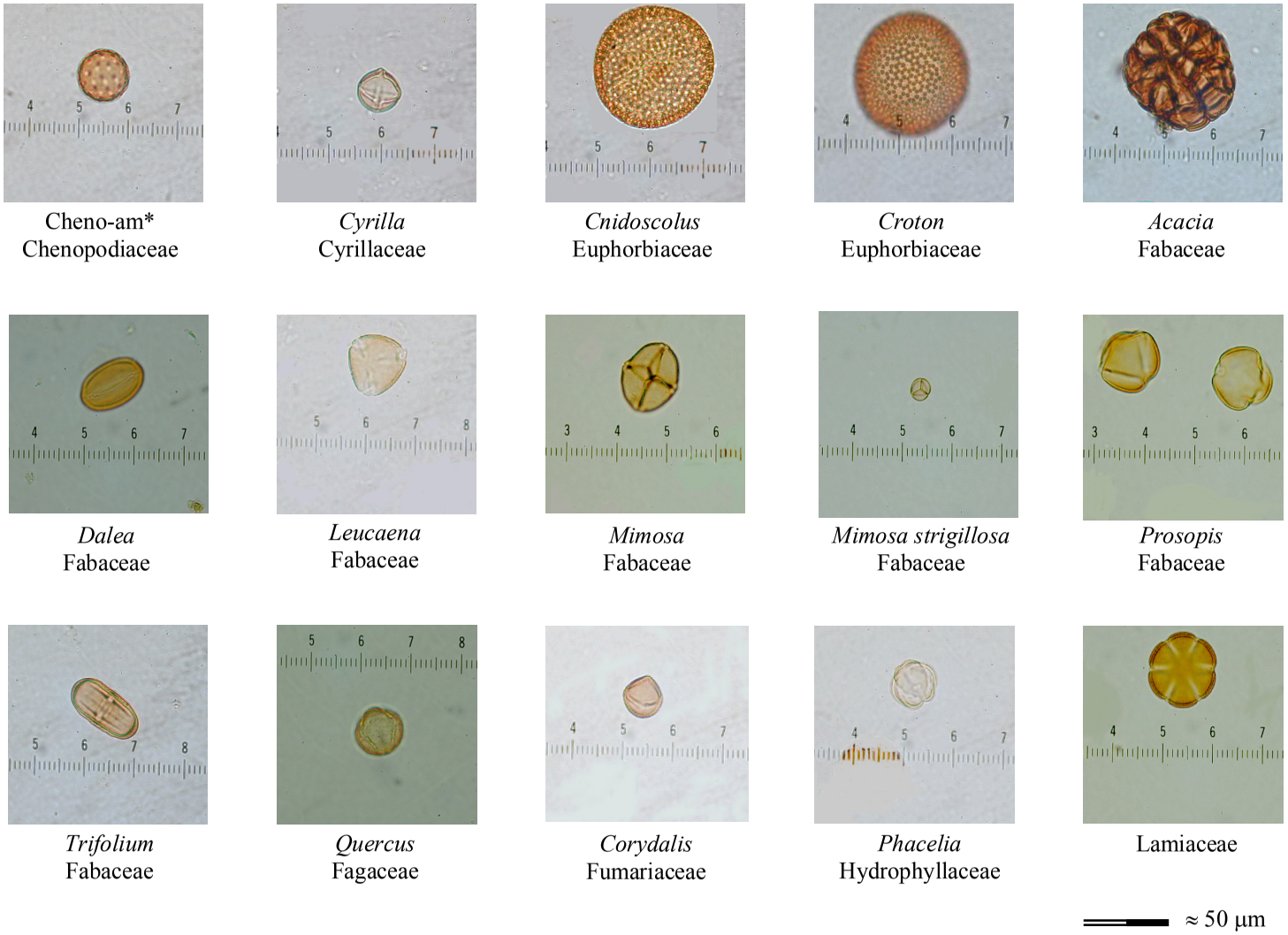
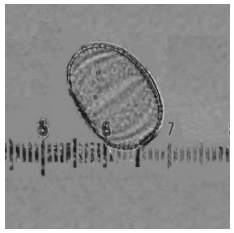


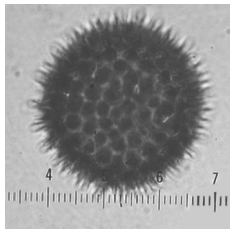

Figure 40. Continued.



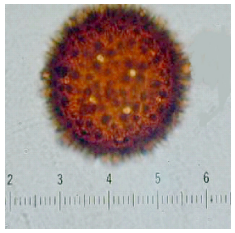
*Salvia*
Lamiaceae

*Schoenocaulon*
Liliaceae

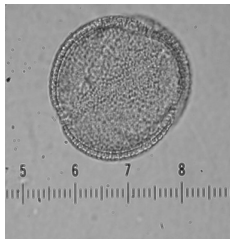
*Lythrum*
Lythraceae



Malvaceae

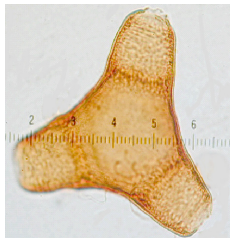


Nyctaginaceae

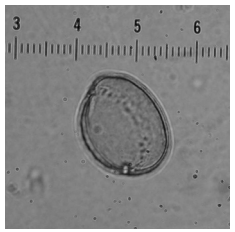


*Nelumbo*
Nymphaceae

*Fraxinus*
Oleaceae

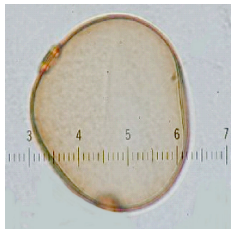


*Oenothera**
Onagraceae

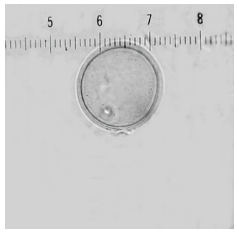


*Argemone*
Papaveraceae

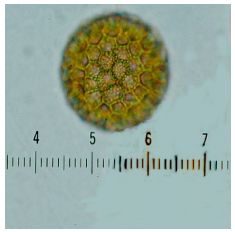
*Plantago*
Plantaginaceae



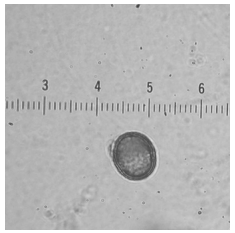
*Zea mays*
Poaceae



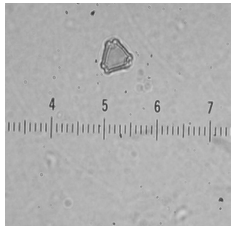
Poaceae



*Polygonum*
Polygonaceae



Ranunculaceae



Rhamnaceae

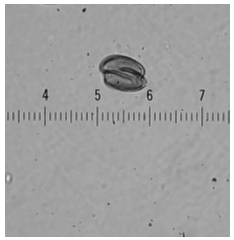
* smaller than actual size

≈ 50 µm

Figure 40. Continued.

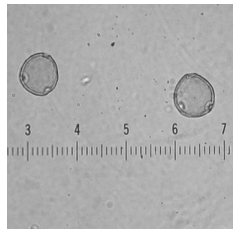
*Prunus*
Rosaceae

*Salix*
Salicaceae

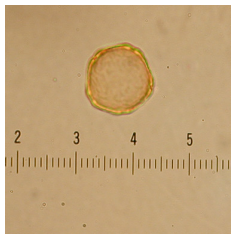


*Pedicularis*
Scrophulariaceae

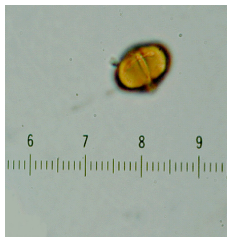
Solanaceae



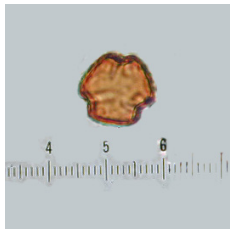
*Celtis*
Ulmaceae



*Ulmus*
Ulmaceae



*Phyla*
Verbenaceae



*Verbena*
Verbenaceae

*Vitis*
Vitaceae

≈ 50 µm

Figure 40. Continued.

Table 21. Shaded areas represent predominant pollen types (> 45 % of a sample from any colony during any sampling period) and numbers represent the percentage of a cumulatively important pollen type (> 8 % of the combined foraging effort of all sampled colonies for a given sampling period) for a given time period.

| | Feb 18 | Mar 11 | Apr 1 | Apr 21 | May 13 | May 31 | Jun 22 | Jul 14 | Aug 2 | Aug 18 | Sep 9 | Sep 30 | Oct 21 | Nov 11 | Dec 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Anacardaceae | | | | | | | | | | | | | | | |
| Anacardaceae *Rhus* I | 8.15 | 38.19 | 8.01 | | 60.34 | | | | | | | | | | |
| Anacardaceae *Rhus* II | | 33.65 | 12.45 | 8.98 | 9.13 | | | | | | | | | | |
| Apiaceae | | | | | | 22.40 | 33.60 | | | | | | | | |
| Arecaceae | | | | | | | | 21.30 | | | | | | | |
| Asteraceae (high spine) | | | | 15.10 | | | | | | | 13.70 | | | 14.71 | 10.53 |
| Asteraceae (low spine) | | | 37.11 | | | | | | | | | 81.71 | | 57.75 | 74.27 |
| Cyrillaceae *Cyrilla* | | | | | | | | | | | | | | | |
| Euphorbiaceae *Cnidoscolus* | | | | | | | | | | 8.21 | | | | | |
| Euphorbiaceae *Croton* | | | | | | | | | | 33.03 | 72.51 | | | | |
| Fabaceae *Mimosa* | | | | | | | | 8.01 | | | | | | | |
| Fabaceae *Prosopis* | | | | | | 19.84 | 56.91 | 61.91 | 76.24 | | | | 29.89 | | |
| Lamiaceae | 37.42 | | 10.92 | | | | | | | | | | | | |
| Lamiaceae *Salvia* | 8.55 | | | | | | | | | | | | | | |
| Lythraceae *Lythrum* | | | | 40.36 | | | | | | | | | | | |
| Papaveraceae *Argemone* | | | | 9.46 | | | | | | | | | | | |
| Poaceae | | | | | | 10.52 | | | | | | | | | |
| Rhamnaceae | | | | | | | | | | 8.56 | | | | | |
| Saliaceae *Salix* | | 10.82 | 8.85 | | | | | | | | | | | | |
| Solanaceae | | | | | | | | | | | | | | | |
| Ulmaceae *Celtis* | | | | | | | | | | 15.43 | | 8.47 | 53.52 | | |
| Unk tetra colporate | 33.31 | | | | | | | | | | | | | | |

high spine Asteraceae, *Cyrilla*, Cnidoscolus, *Prosopis*, Rhamnaceae, and Solanaceae. The winter collection period consisted of a dearth of pollen availability and little foraging activity from late December through January, with Lamiaceae, an unknown pollen type, *Salvia*, and *Rhus* I dominating in mid February (Table 21).

Significant differences existed in the amount of overlap between colonies among many of the sampling periods (Table 22). The amount of overlap and distance between colonies were significantly correlated for some of the sampling periods (Mar 11, Apr 21, Aug 2, Aug 18, Sep 9, and Oct 21), but not for others (Feb 18, Apr 1, May 13, May 31, Jun 22, Jul 14, Sep 30, Nov 11, and Dec 11) (Table 23). The pollen types collected during a sampling period were typically located in multiple habitat types that were distributed throughout the study area (Table 24, Figure 39). Therefore, collected pollen types could not be associated with specific spatial locations or foraging distances.

Protein content values were obtained for 17 of the 22 predominant (> 45 % of a sample from any colony during any sampling period) and cumulatively important pollen types (> 8 % of the combined foraging effort of all sampled colonies for a given sampling period), with a mean and standard deviation of 32.17 ± 8.54 % protein (Table 25). Twelve of these pollen types were overestimated and nine were underestimated. Furthermore, higher than average protein content values increased two underestimations and decreased four overestimations when considering the overall nutritional content of the pollen types. Lower than average protein content values decreased five underestimations and increased six overestimations (Table 25).

Table 22. Means, standard deviations, and p-values from a Wilcoxon signed rank test comparing the overlap of collected pollen types between pairs of colonies and sampling periods.

| | Feb 18 | Mar 11 | Apr 1 | Apr 21 | May 13 | May 31 | Jun 22 | Jul 14 | Aug 2 | Aug 18 | Sep 9 | Sep 30 | Oct 21 | Nov 11 | Dec 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feb 18 | na | 0.9236 | 0.1356 | 0.0553 | 0.0638 | 0.0018 | 0.8813 | 0.5097 | <.0001 | 0.0137 | 0.0001 | <.0001 | 0.0056 | 0.1377 | <.0001 |
| Mar 11 | 0.9236 | na | 0.6262 | 0.0005 | 0.6374 | <.0001 | 0.4553 | 0.925 | <.0001 | 0.0071 | <.0001 | <.0001 | 0.0069 | 0.0076 | <.0001 |
| Apr 1 | 0.1356 | 0.6262 | na | 0.001 | 0.4796 | <.0001 | 0.5257 | 0.3967 | <.0001 | 0.0011 | <.0001 | <.0001 | 0.0072 | 0.1126 | <.0001 |
| Apr 21 | 0.0553 | 0.0005 | 0.001 | na | <.0001 | 0.1717 | 0.0793 | 0.0156 | <.0001 | 0.379 | <.0001 | <.0001 | <.0001 | 0.001 | <.0001 |
| May 13 | 0.0638 | 0.6374 | 0.4796 | <.0001 | na | 0.0002 | 0.5755 | 0.5936 | <.0001 | 0.0162 | <.0001 | 0.0003 | 0.0278 | 0.5717 | <.0001 |
| May 31 | 0.0018 | <.0001 | <.0001 | 0.1717 | 0.0002 | na | 0.0152 | 0.0355 | <.0001 | 0.0038 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Jun 22 | 0.8813 | 0.4553 | 0.5257 | 0.0793 | 0.5755 | 0.0152 | na | 0.9249 | 0.0013 | 0.6542 | 0.0013 | 0.0152 | 0.179 | 0.3507 | 0.0015 |
| Jul 14 | 0.5097 | 0.925 | 0.3967 | 0.0156 | 0.5936 | 0.0355 | 0.9249 | na | 0.0023 | 0.3305 | 0.0029 | 0.0413 | 0.0413 | 0.7299 | 0.0035 |
| Aug 2 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0013 | 0.0023 | na | <.0001 | 0.1738 | 0.0666 | 0.0002 | 0.0027 | 0.0363 |
| Aug 18 | 0.0137 | 0.0071 | 0.0011 | 0.379 | 0.0162 | 0.0038 | 0.6542 | 0.3305 | <.0001 | na | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| Sep 9 | 0.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0013 | 0.0029 | 0.1738 | <.0001 | na | 0.0216 | 0.006 | 0.1102 | 0.0132 |
| Sep 30 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0003 | <.0001 | 0.0152 | 0.0413 | 0.0666 | <.0001 | 0.0216 | na | 0.0004 | <.0001 | 0.6234 |
| Oct 21 | 0.0056 | 0.0069 | 0.0072 | <.0001 | 0.0278 | <.0001 | 0.179 | 0.0413 | 0.0002 | <.0001 | 0.006 | 0.0004 | na | 0.7735 | 0.0001 |
| Nov 11 | 0.1377 | 0.0076 | 0.1126 | 0.001 | 0.5717 | <.0001 | 0.3507 | 0.7299 | 0.0027 | <.0001 | 0.1102 | <.0001 | 0.7735 | na | <.0001 |
| Dec 11 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0015 | 0.0035 | 0.0363 | <.0001 | 0.0132 | 0.6234 | 0.0001 | <.0001 | na |
| mean ± std dev | 42.53 ± 22.59 | 43.05 ± 26.98 | 48.29 ± 15.07 | 34.74 ± 19.24 | 50.21 ± 20.67 | 29.27 ± 18.12 | 50.86 ± 24.48 | 55.93 ± 21.30 | 69.40 ± 13.72 | 31.87 ± 15.85 | 65.12 ± 21.44 | 75.85 ± 21.02 | 56.23 ± 23.35 | 53.42 ± 30.96 | 75.31 ± 12.51 |

Table 23. Means, standard deviations, rho values, and p-values from a Spearman rank test for correlation between pollen overlap and distance between colonies. The mean number of pollen types (averaged across all colonies), total number of pollen types (summed across all colonies), number of predominant pollen types (> 45 % of a sample from any colony), and number of colonies without a predominant pollen type are provided for each sampling period.

| | | | | pollen types | | | |
|---|---|---|---|---|---|---|---|
| | overlap mean ± stdev | rho | p-value | mean number | total number | number predominant | number of colonies without a predominant |
| Feb 18 | 42.53 ± 22.59 | -0.038 | 0.803 | 6.4 | 17 | 3 | 3 |
| Mar 11 | 43.05 ± 26.98 | -0.455 | 0.003 | 6.4 | 21 | 3 | 2 |
| Apr 1 | 47.86 ± 16.87 | 0.241 | 0.153 | 12 | 24 | 1 | 6 |
| Apr 21 | 30.92 ± 19.76 | -0.353 | 0.037 | 7 | 19 | 5 | 2 |
| May 13 | 50.26 ± 23.18 | -0.271 | 0.110 | 8.1 | 27 | 2 | 0 |
| May 31 | 24.08 ± 16.57 | 0.091 | 0.591 | 6. 6 | 20 | 2 | 6 |
| Jun 22 | 41.93 ± 28.19 | -0.03 | 0.895 | 3.1 | 10 | 3 | 0 |
| Jul 14 | 44.07 ± 16.52 | -0.405 | 0.145 | 2.3 | 8 | 2 | 0 |
| Aug 2 | 69.40 ± 13.72 | -0.551 | <.001 | 5.6 | 16 | 1 | 6 |
| Aug 18 | 31.87 ± 15.85 | -0.327 | 0.03 | 8.2 | 23 | 2 | 0 |
| Sep 9 | 65.12 ± 21.44 | -0.465 | 0.002 | 7.1 | 25 | 2 | 0 |

Table 23. Continued.

| | | | | pollen types | | | |
|---|---|---|---|---|---|---|---|
| | overlap mean ± stdev | rho | p-value | mean number | total number | number predominant | number of colonies without a predominant |
| Sep 30 | 75.85 ± 21.02 | 0.277 | 0.066 | 6.3 | 16 | 2 | 0 |
| Oct 21 | 56.23 ± 23.35 | -0.549 | <.001 | 6.8 | 14 | 2 | 1 |
| Nov 11 | 53.42 ± 30.96 | 0.096 | 0.522 | 6.8 | 22 | 2 | 2 |
| Dec 11 | 75.31 ± 12.51 | 0.027 | 0.855 | 5 | 15 | 2 | 0 |

Table. 24. The abundance of predominant and cumulatively important pollen types in different habitat types within the study area based on data from point frame transects provided in the Welder plant list (unpublished data, Welder Wildlife Foundation). Abundance values corresponded to cover data, with abundant species (A) = > 5 % cover, frequent species (F) = > 1-5 % cover, occasional species (O) = 0-1 % cover, and rare species (R) = not encountered during sampling, but seen along sampling transect.

| | brushland | live oak | woodland | aquatic | disturbed |
|---|---|---|---|---|---|
| Anacardaceae | | O | O | | |
| Anacardaceae *Rhus* I | | O | F | | |
| Anacardaceae *Rhus* II | | O | | | |
| Apiaceae | O | O | | | O |
| Arecaceae | | | R | | |
| Asteraceae (high spine) | A | A | A | | A |
| Asteraceae (low spine) | F | O | | | O |
| Cyrillaceae *Cyrilla* | | | | R | |
| Euphorbiaceae *Cnidoscolus* | | O | | | |
| Euphorbiaceae *Croton* | O | A | O | | F |
| Fabaceae *Mimosa* | F | | F | | |
| Fabaceae *Prosopis* | A | A | O | | |
| Lamiaceae | O | O | | | |
| Lamiaceae *Salvia* | | O | | | |
| Lythraceae *Lythrum* | O | | | | |
| Papaveraceae *Argemone* | | O | | | O |
| Poaceae | A | A | A | A | A |
| Rhamnaceae | O | F | F | | |
| Saliaceae *Salix* | | | O | | |
| Solanaceae | O | F | O | O | R |
| Ulmaceae *Celtis* | F | O | F | | |

Table 25. Predominant (> 45 % of a sample from any colony during any sampling period) and cumulatively important (> 8 % of the combined foraging effort of all sampled colonies for a given sampling period) pollen types with overestimated and underestimated contributions based on volume. The proportion by volume, proportion by abundance, and proportional difference are based on the contribution of a pollen type to the overall sample (summed across all sampling periods and all colonies). The result (overestimated or underestimated), protein content, and difference from the mean protein content are provided.

| pollen type | proportion by volume | proportion by abundance | proportional difference | result | protein content* | difference from mean** |
|---|---|---|---|---|---|---|
| Anacardiaceae | 3.00 | 4.13 | 0.73 | overestimated | 28.93 | -3.24 |
| Anacardaceae *Rhus* I | 2.38 | 5.58 | 0.43 | overestimated | 28.93 | -3.24 |
| Anacardaceae *Rhus* II | 3.62 | 2.68 | 1.35 | underestimated | 28.93 | -3.24 |
| Apiaceae | 1.26 | 2.96 | 0.43 | overestimated | 29.00 | -3.17 |
| Arecaceae | 0.90 | 0.67 | 1.35 | underestimated | 31.40 | -0.77 |
| Asteraceae (high spine) | 2.04 | 4.79 | 0.43 | overestimated | 20.85 | -11.32 |
| Asteraceae (low spine) | 7.95 | 18.66 | 0.43 | overestimated | 24.74 | -7.43 |
| Cyrillaceae *Cyrilla* | 0.20 | 0.47 | 0.43 | overestimated | na | na |
| Euphorbiaceae *Cnidoscolus* | 2.59 | 0.51 | 5.04 | underestimated | na | na |
| Euphorbiaceae *Croton* | 36.26 | 7.19 | 5.04 | underestimated | na | na |

Table 25. Continued.

| pollen type | proportion by volume | proportion by abundance | proportional difference | result | protein content* | difference from mean** |
|---|---|---|---|---|---|---|
| Fabaceae *Mimosa* | 0.04 | 0.03 | 1.35 | underestimated | 43.34 | 11.18 |
| Fabaceae *Prosopis* | 11.83 | 27.77 | 0.43 | overestimated | 39.00 | 6.83 |
| Lamiaceae | 6.41 | 4.75 | 1.35 | underestimated | 22.80 | -9.37 |
| Lamiaceae *Salvia* | 1.44 | 1.07 | 1.35 | underestimated | 22.80 | -9.37 |
| Lythraceae *Lythrum* | 1.22 | 2.87 | 0.43 | overestimated | na | na |
| Papaveraceae *Argemone* | 1.26 | 0.93 | 1.35 | underestimated | 45.30 | 13.13 |
| Poaceae | 0.75 | 0.55 | 1.35 | underestimated | 25.26 | -6.91 |
| Rhamnaceae | 0.04 | 0.62 | 0.07 | overestimated | 40.40 | 8.23 |
| Saliaceae *Salix* | 0.05 | 0.74 | 0.07 | overestimated | 40.84 | 8.67 |
| Solanaceae | 0.40 | 0.94 | 0.43 | overestimated | 46.71 | 14.54 |
| Ulmaceae *Celtis* | 3.17 | 7.45 | 0.43 | overestimated | 27.65 | -4.52 |

na = not available

* protein content values from Roulston et al. (2000)

** difference from mean protein content of 32.17

Entomophilous pollen types were important for most of the year, while anemophilous pollen types were important from late September through December (Figure 41). The use of herbaceous and woody pollen sources fluctuated throughout the year, with herbs and shrubs being important pollen sources at the beginning of the year, trees from late June through early August, and herbs again for the remainder of the year (Figure 42). In general, pollen sources were also known honey plants (Figure 43).

## DISCUSSION

The colonies collected a wide variety of pollen types throughout the year, comprising about 30 % of the species on the Welder plant list (unpublished data, Welder Wildlife Foundation). Other research also suggests honey bees typically collect pollen from 20 to 30 % of angiosperms within their foraging range, although few of these species are used intensively (Wills et al. 1990, Roubik 1991, Buchmann et al. 1992).

Overall, the most important pollen sources included low spine Asteraceae, *Prosopis*, *Rhus* I, *Croton*, high spine Asteraceae, *Celtis*, *Rhus* II, Lamiaceae, Apiaceae, *Lythrum*, and an unknown pollen type. However, the timing of pollen collection patterns may be as important or more important than the amount any given pollen type contributes to the annual pollen harvest of a colony (O'Neal and Waller 1984). Pollen types collected during periods of low pollen availability and/or early in the foraging season when brood rearing begins may be functionally more important than those collected at other times when pollen availability is high and brood rearing is well established.

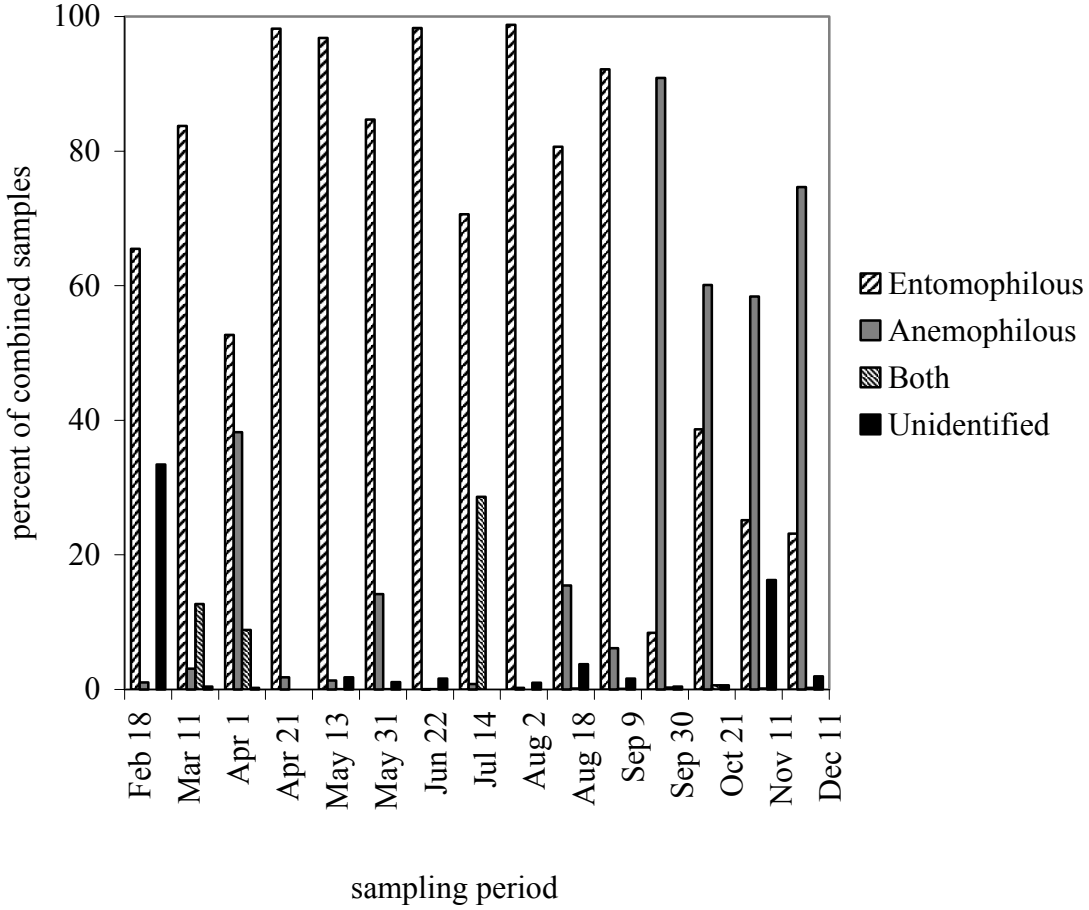



Figure 41. Percent of entomophilous, anemophilous, both (entomophilous and anemophilous), and unidentified pollen types collected each sampling period.

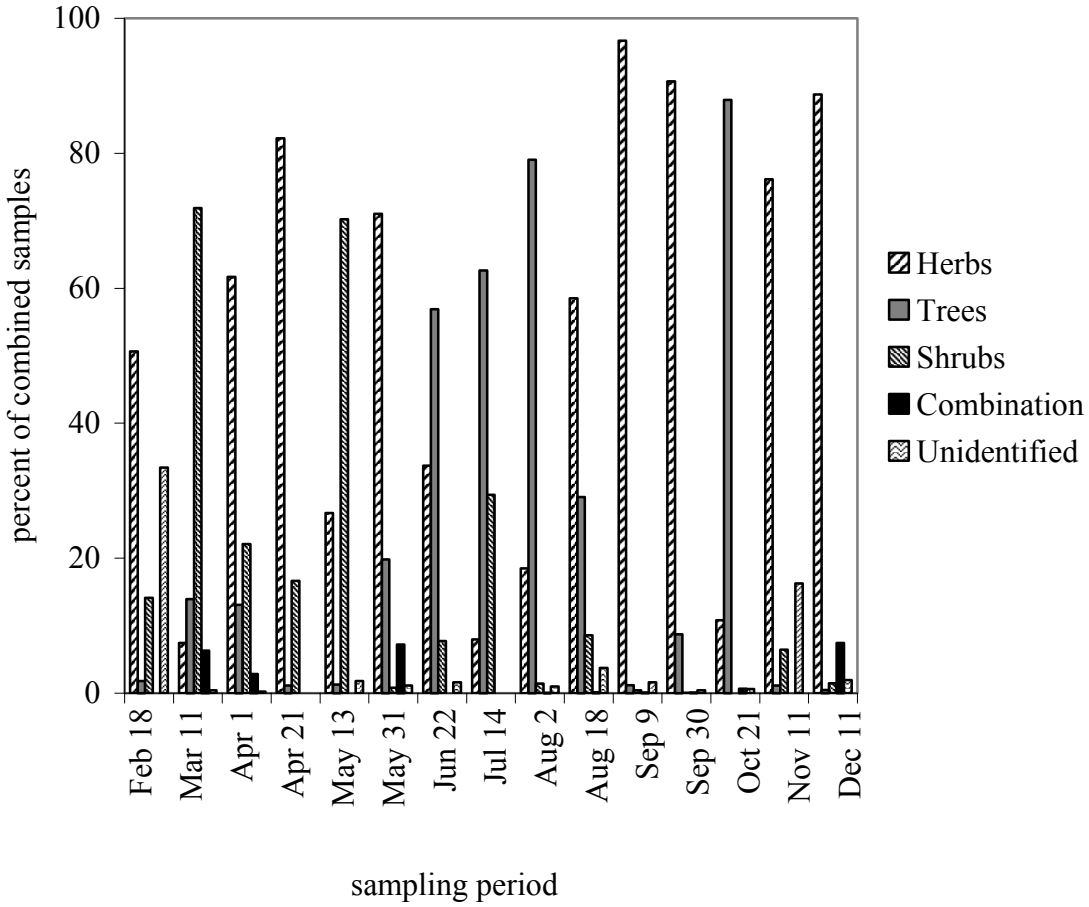



Figure 42. Percent of pollen types from herbs, trees, shrubs, combination (pollen types that include both herbaceous and woody plants at the level of identification), and unidentified pollen sources collected each sampling period.

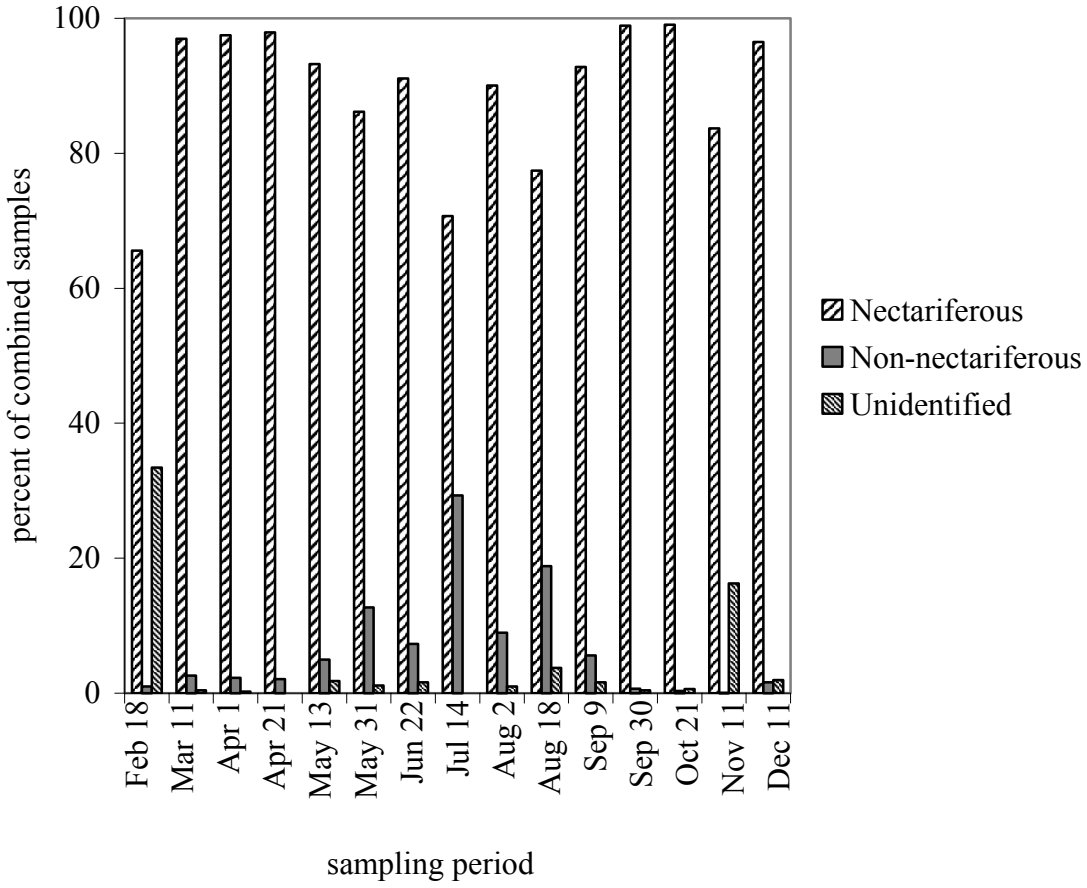



Figure 43. Percent of pollen types from nectariferous, non-nectariferous, and unidentified pollen sources collected each sampling period.

Characteristics of the pollen types varied in terms of the length of collection periods, the importance within a sampling period, and protein content. Some pollen types were collected throughout much of year. For example, low spine Asteraceae comprised 21.4 % of all samples combined (all colonies and all sampling periods), which was twice as much as the next common pollen type. Low spine Asteraceae also occurred in all but two of the sampling periods (Feb. 18 and Aug. 18), and was a predominant pollen type in November and December when pollen availability was relatively low. However, low spine Asteraceae pollen also contained less protein than many of the other pollen types. In contrast, *Prosopis* was collected in all but three sampling periods (Feb. 18, Mar. 11, and Dec. 11), represented a predominant pollen type in five sampling periods (the most of any pollen type), and contained a relatively high protein content. Other pollen types were collected for only brief periods of time. *Lythrum* was collected during a two-month period from mid April to mid June and was a predominant pollen type during only one of the sampling periods.

Solanaceae, *Argemone*, and *Mimosa* contained the highest protein contents, followed by *Salix*, Rhamnaceae, and *Prosopis* (Table 25). High spine Asteraceae contained the lowest protein content, followed by Lamiaceae, *Salvia*, low spine Asteraceae, and Poaceae. The protein content and volume of a pollen type may change its overall nutritional contribution to the diet of a feral honey bee colony compared to the frequency of the pollen type (da Silveira 1991, O'Rourke and Buchmann 1991). For example, the contribution of a very abundant, but also very small pollen grain may be overestimated by evaluating only the percentage that pollen type comprises of a sample.

At the other extreme, the value of an uncommon, but extremely large pollen grain may be underestimated by only examining frequencies.

Pollen types with low and high protein contents may be even more overestimated or underestimated when overall nutritional contribution is considered. Although evaluating the nutritional content of different pollen types is beyond the scope of this project, considering general characteristics of pollen volume and protein content may provide additional insights into the observed patterns of pollen collection. The normal procedure for estimating pollen volume involves measuring numerous pollen grains of each species using reference slides made from pollen collected from vouchered plant specimens (O'Rourke and Buchmann 1991). However, this was not possible since pollen types were only identified to the genus or family level, with a few exceptions.

The value of several of the pollen types was misrepresented based on abundance alone. Most notably, the value of *Cnidoscolus* and *Croton* was underestimated and the value of Rhamnaceae and *Salix* was overestimated. Protein content values were not available for *Cnidoscolus* and *Croton*, but Rhamnaceae and *Salix* had higher than average protein contents, which would increase their value.

In general, four main foraging periods were identified from the pollen collection patterns of feral colonies on the Welder Wildlife Refuge. The spring, summer, and winter periods consisted mainly of entomophilous pollen types, while anemophilous pollen types were important from late September through December (Figure 41). Other studies have also identified brief periods when anemophilous pollen types were important to honey bee colonies (reviewed in O'Neal and Waller 1984, Pearson and

Braiden 1990). Depending on geographic location and local plant communities, anemophilous pollen can make up 0.5 % to 71 % of the annual pollen harvest of a colony (Percival 1947, O'Neal and Waller 1984).

This periodic focus on anemophilous pollen types is interesting because these plants do not possess characteristics that attract insects for the dispersal of pollen, but instead rely on the wind to provide pollination services. In terms of honey bee colonies, the collection of anemophilous pollens requires nectar or honey to provide energy for foraging and to pack pollen grains into corbicular pellets (O'Neal and Waller 1984). However, protein content does not differ between zoophilous and anemophilous pollen types when phylogeny is considered (Roulston et al. 2000), although honey bee development based on these different pollen types was not considered. Futhermore, Roulston et al. (2000) found that bees do not appear to select pollen types based on protein content. Therefore, honey bees may collect a variety of pollen types to meet their pollen needs, increasing the probability of obtaining nutritionally suitable pollens (Gary et al. 1972). Collecting numerous pollen types should also decrease the chance of poisoning by consuming a toxic pollen type or nutritional imbalances due to the consumption of only one pollen type (O'Neal and Waller 1984).

The use of herbs, trees, and shrubs as pollen sources fluctuated throughout the year. Herbs and shrubs were important during the spring and early summer, trees were important during the mid to late summer, and herbs were important during the fall, roughly corresponding to the identified foraging periods (Figure 42). Different patterns are observed in different locations (Severson and Parry 1981, Coffey and Breen 1997).

For example, trees provided the initial flow of pollen in the spring, while herbs provided the majority of pollen for the remainder of the foraging season in Wisconsin (Severson and Parry 1981). Pollen sources were also good honey plants for most of the year, although the summer collection period contained the least correspondence between nectar and pollen sources (Figure 43).

The foraging range of a colony varies depending on a number of factors related to resource availability and colony status. Foraging distances reported in the literature range from a few meters to over 10000 m (Gary et al. 1972, Visscher and Seeley 1982, Schneider 1989, Schneider and McNally 1993, Waddington et al. 1994, Schneider and Hall 1997, Beekman and Ratnieks 2000). For example, Visscher and Seeley (1982) recorded a mean foraging distance of 2260 m for a colony located in a temperate forest in New York, with distances ranging from 50 m to 10100 m. Gary et al. (1972) reported mean foraging distances of 557 m and 1663 m in an agricultural setting, with distances ranging from 41 m to 6117 m. Therefore, all the colonies within the 2500 m by 2500 m area used in this study were within the potential foraging ranges of all the other colonies and all the floral patches within the study area were available to all the colonies.

No clear patterns emerged from the overlap in pollen use between sampling periods (Table 22). Thus, the colonies did not use the same resources in the same percentages, or exploit resources in a consistent manner throughout the year. This emphasizes both the complexity of honey bee foraging behavior and the flowering phenology in the study area. Honey bee colonies choose to collect pollen from a variety of potential sources based on incomplete knowledge of the resources available within

their foraging range. The extent of a colony's knowledge probably varies with resource availability, with the colonies obtaining more complete knowledge about the resources within their foraging range when resource availability is low (Waddington et al. 1994). The Welder Wildlife Refuge provided a wide variety of pollen sources that were typically abundant within the study area for most of the year. Thus, the colonies would be expected to have less complete knowledge of the available resources and potentially utilize resources in different ways.

Other studies have documented colonies concentrating their foraging effort on a small number of large patches (Visscher and Seeley 1982, Schneider 1989) or a relatively large number of small, rich patches (Waddington et al. 1994), depending on the distribution and availability of floral resources in the area. Although no information was available on the number of patches visited in this study, the number of collected pollen types was known (Table 23). The mean number of pollen types collected showed that the colonies probably focused on the largest number of patches in late March and early April, and the fewest patches from mid June to mid July. Furthermore, the number of colonies without predominant pollen types indicated the colonies concentrated their foraging effort on several different sources in late March and early April, late May and early June, and late July and early August. Interestingly, the other sampling periods from mid May through late September were marked with all colonies collecting at least one predominant pollen type.

The significant correlation between overlap in collected pollen types and distance between colonies for some sampling periods suggested localized patterns of pollen

availability were important at certain times of the year (Table 23). At other times, larger scale patterns (at a broad spatial extent) appeared to drive pollen collection, and no correlation was found between overlap and distance. However, these patterns could be created by a number of different scenarios. For example, no correlation may exist when resources are scarce and the colonies must utilize the same resources, regardless of the distance from the hive. At the other extreme, there may be no correlation between overlap and distance when important pollen sources are abundant and widely distributed.

Another interesting observation is that colonies located only a few meters apart often collect very different pollen types (Waddington et al. 1994, unpublished data, K. A. Baum, W. L. Rubink, and R. N. Coulson). Again, this can be attributed to the sampling effort of the colony and the way in which a colony chooses among available resources.

In conclusion, feral colonies collected a wide variety of pollen types in a coastal prairie landscape. Four main foraging periods were identified, with anemophilous pollen types being important in the fall. Herbaceous plants and shrubs provided pollen during the spring and early summer, trees in mid to late summer, and herbaceous plants in the fall. The pollen sources utilized by the feral colonies also tended to be good nectar sources. Overlap in pollen use between colonies varied throughout the year and pollen overlap was correlated with distance for some sampling periods and not others, probably due to the way colonies choose among resources and the flowering phenology in the study area.

# CHAPTER V

# RESOURCE USE – AERIAL PITFALL TRAPS

## INTRODUCTION

Honey bee colonies contain thousands of individuals that must coordinate their activities to select among a variety of resources. Colonies select nest sites when swarming or absconding, pollen and nectar sources when foraging, and water sources when collecting water. A subset of a colony's foragers searches for and finds potential nest sites and food sources (Seeley 1983, Gilley 1998). They return to the colony and perform dances that code information about resource distance, direction, and quality. Other workers in the colony then recruit to these resources in varying numbers.

Nest site selection differs from the selection of food sources in several ways (Camazine et al. 1999). Multiple foraging sites are utilized at the same time, whereas only one nest site is used (Visscher and Seeley 1982, Schneider 1989, Waddington et al. 1994). This behavior translates into different recruitment systems. Multiple foraging sites are advertised and several to many are utilized simultaneously. Many nest sites are advertised initially, but the selection process continues until a consensus is reached and a single site is selected. These recruitment systems have implications for quality selection, since not all foragers select the best pollen or nectar source, but presumably the best nest site is selected (Camazine et al. 1999). Seeley and Buhrman (2001) found that swarms usually selected the best nest box. However, the time of discovery and subsequent

length of recruitment time for a nest site, as well as variability in the dancing intensity of individual bees, add randomness to the process (Camazine et al. 1999).

The behavior of bees searching for resources has been studied under controlled conditions using managed colonies (Seeley 1983, Seeley and Buhrman 1999, 2001). However, it is more difficult to study the behavior of bees in feral colonies under natural conditions, since dancing bees cannot be observed inside a tree cavity. It is also difficult to examine the behavior of bees searching for resources at the population level, since an observer would be needed at every colony. Therefore, the goal of this study was to evaluate the behavior of honey bee scouts[5] for a population of feral honey bee colonies. Specific objectives included examining search rates for resources throughout the year and comparing search rates in different habitats.

## METHODS

The study site was located on the Welder Wildlife Refuge in San Patricio County, Texas, where there is a dense population of feral honey bee colonies. Over a 12-year period, 109 cavities were identified that contained feral colonies. During the time period of this study, 56 to 64 of those cavities were occupied.

I used aerial pitfall traps to estimate the number of honey bee searching for

[5] Typically, the term scout has been used to refer to individual bees that independently search for and find new food sources or nest sites (Seeley 1985, Winston 1987, Biesmeijer and de Vries 2001). Recruit has been used to indicate individual bees that use information provided by others to find an advertised resource. These terms are confusing because scouts may not find a new resource and recruits may find different resources from those advertised in the dance (Seeley 1983, Biesmeijer and de Vries 2001). For the purposes of this study, a scout can be defined as an individual that finds a new resource, including novice bees, experienced but unemployed foragers, and lost recruits (Biesmeijer and de Vries 2001).

resources in a given area. Aerial pitfall traps consisted of 2.1 l plastic jars baited with honey and a 1:1 citral:geraniol mixture, which served as a honey bee attractant by imitating the Nasonov pheromone (Figure 44) (Sugden et al. 1989, Rubink et al. 1990a). Nasonov gland secretions serve many purposes, including the attraction of foragers to high quality food sources and the regulation of swarm movement and formation (Winston 1987). The citral:geraniol mixture was placed in snap-top, 0.4 ml, polyethylene centrifuge tubes, and inserted into 120 ml plastic cups attached to the lid of each trap, which also contained the honey. Honey is a concentrated carbohydrate source that is quickly exploited by foragers when available. Honey bees may even rob the honey stores of other colonies (Winston 1987), therefore, honey is a strong attractant for honey bees searching for nectar sources. Propylene glycol in the bottom of the trap preserved any arthropods that entered the trap for future identification and possible genetic analysis (Post et al. 1993, Reiss et al. 1995, Dillon et al. 1996, Rubink et al. 2003). I placed traps in 40 random locations within a 7.6 $km^2$ area by hanging them from the branches of woody vegetation approximately 1.2 to 1.8 m above ground level, or in areas without woody vegetation by attaching them to a stake at 1.2 m above ground level (Figure 45). Random locations were selected by placing a 50-meter grid over a mosaicked image of the study area, and then using computer generated random numbers to select grid cells for sampling. Random locations were navigated to using a Trimble GPS Pathfinder™ receiver and TSC1™ Asset Surveyor™ data logger, with the coordinates of the center point of each randomly selected grid cell. Traps were baited at three-week intervals from July 2000 through July 2001, so each sample consisted of

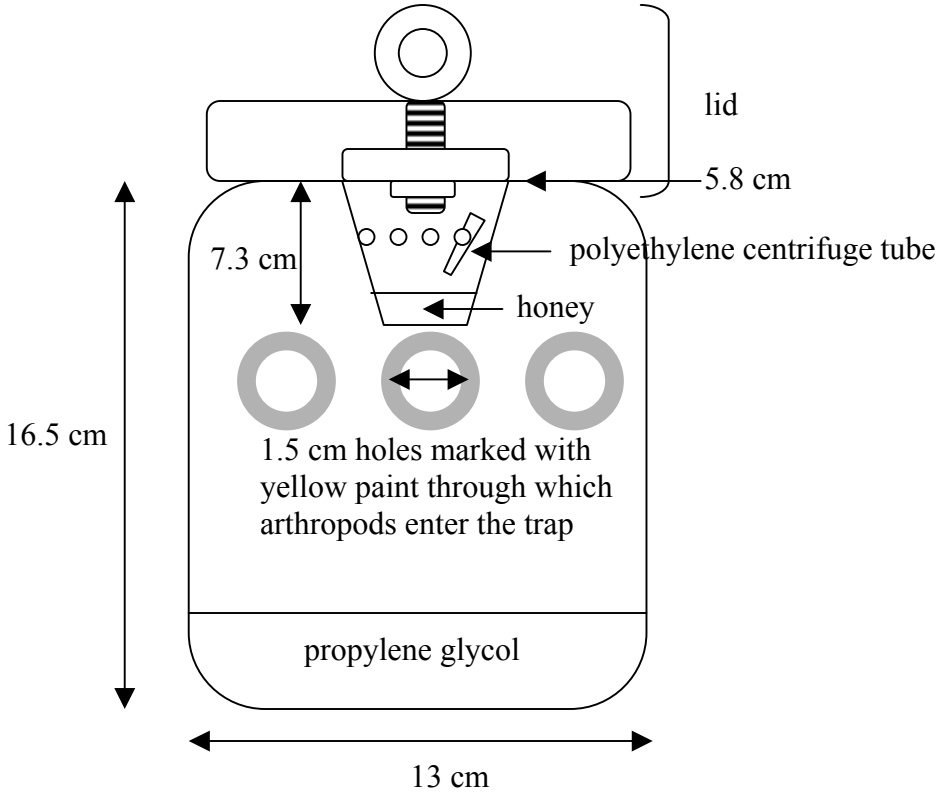



Figure 44. Aerial pitfall trap design. The lid consists of the trap lid with an eye bolt (1/4 x 2-1/2) through center and the bait cup lid attached.

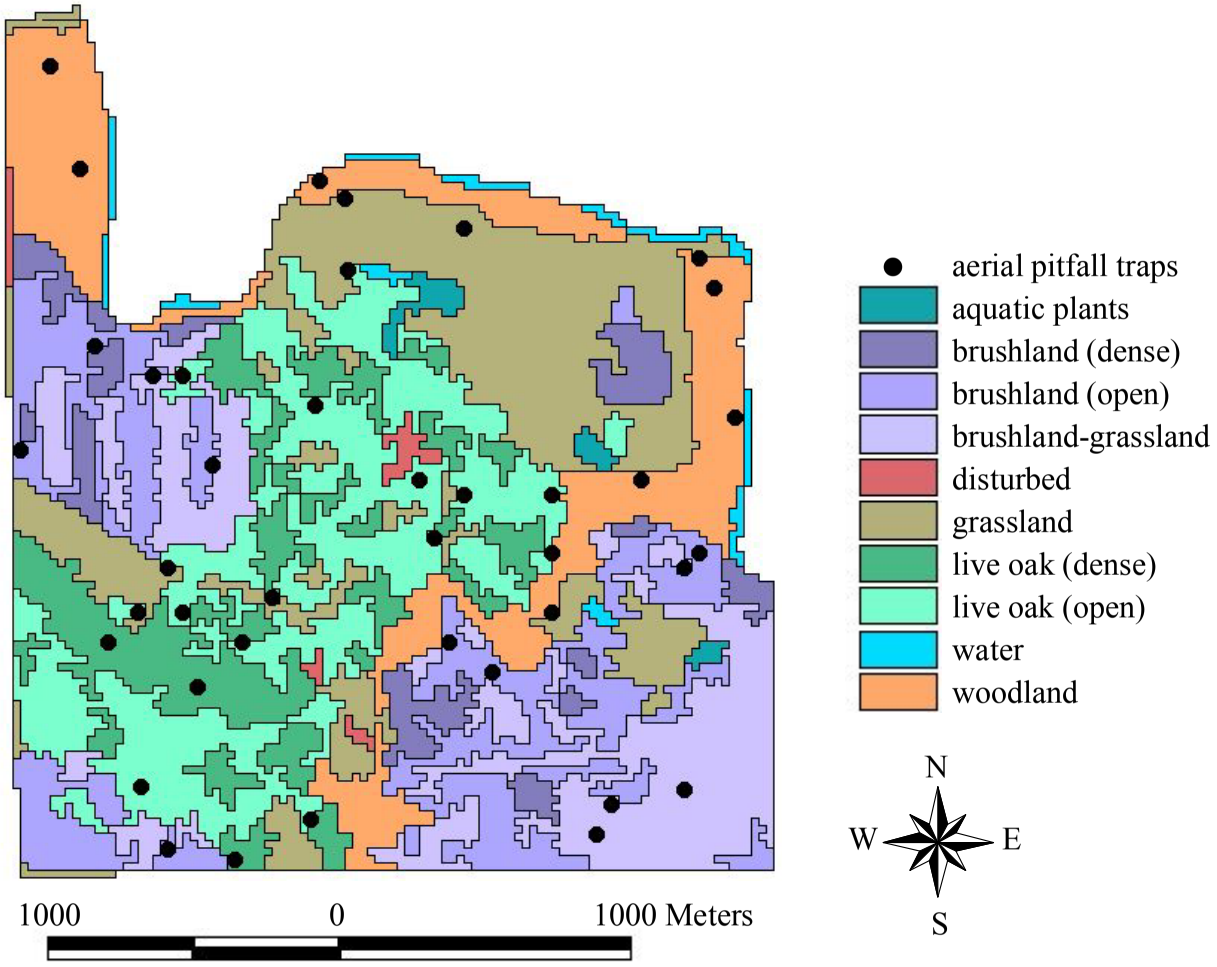



Figure 45. Location of aerial pitfall traps within each habitat type on the Welder Wildlife Refuge.

honey bees collected during a three-week period, followed by a three-week period when no insects were collected.

The number of honey bees collected in each trap was counted. Wilcoxon signed rank tests were used to compare the number of honey bees collected in the traps between sampling periods. The traps were classified by habitat type using a landscape classification of the study area based on vegetation communities (unpublished data, K. A. Baum, W. L. Rubink, and R. N. Coulson). The numbers of honey bees collected in the traps in different habitat types were compared using Kruskal-Wallis and Mann-Whitney *U* tests.

## RESULTS

Search rates differed throughout the year as shown by the number of honey bees collected in the traps (Table 26). The observed patterns could be summarized into time periods with similar search rates, including December through February, March through May, June through August, and September through November. The highest search rates occurred from December through February, and the lowest search rates from June through August. These search rates corresponded to levels of nectar and pollen availability, with a greater number of honey bees being collected in the traps when pollen and nectar availability were low (Figure 46). Search rates also differed between habitats (Table 27, Figure 47). The habitats fell into a general hierarchy, with grassland typically being searched the most frequently and woodland typically being searched the least often.

Table 26. Means, standard deviations, Z-values, and p-values for Wilcoxon signed rank tests comparing the number of honey bees collected per trap between sampling periods. Sampling dates represent the end of a three-week sampling period.

| | | Feb 17 | Apr 1 | May 13 | Jun 22 | Jul 10 | Aug 19 | Oct 1 | Nov 11 | Dec 31 |
|---|---|---|---|---|---|---|---|---|---|---|
| Feb 17 | Z-value | | -5.304 | -5.415 | -5.374 | -5.416 | -5.445 | -5.375 | -5.215 | -1.438 |
| | p-value | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.1506 |
| Apr 1 | Z-value | | | -0.704 | -2.631 | -3.237 | -2.822 | -2.068 | -3.435 | -5.154 |
| | p-value | | | 0.4811 | 0.0085 | 0.0012 | 0.0048 | 0.0386 | 0.0006 | <.0001 |
| May 13 | Z-value | | | | -2.499 | -1.273 | -1.795 | -2.927 | -3.343 | -5.123 |
| | p-value | | | | 0.0125 | 0.2028 | 0.0726 | 0.0034 | 0.0008 | <.0001 |
| Jun 22 | Z-value | | | | | -0.568 | -0.639 | -3.728 | -4.376 | -5.101 |
| | p-value | | | | | 0.5700 | 0.5230 | 0.0002 | <.0001 | <.0001 |
| Jul 10 | Z-value | | | | | | -0.449 | -3.679 | -4.477 | -5.233 |
| | p-value | | | | | | 0.6533 | 0.0002 | <.0001 | <.0001 |
| Aug 19 | Z-value | | | | | | | -3.703 | -4.584 | -5.206 |
| | p-value | | | | | | | 0.0002 | <.0001 | <.0001 |
| Oct 1 | Z-value | | | | | | | | -1.531 | -4.872 |
| | p-value | | | | | | | | 0.1258 | <.0001 |
| Nov 11 | Z-value | | | | | | | | | -4.854 |

Table 26. Continued.

| | | Feb 17 | Apr 1 | May 13 | Jun 22 | Jul 10 | Aug 19 | Oct 1 | Nov 11 | Dec 31 |
|---|---|---|---|---|---|---|---|---|---|---|
| Nov 11 | p-value | | | | | | | | | <.0001 |
| mean | | 27.53 | 0.93 | 1.05 | 0.40 | 0.30 | 0.38 | 2.55 | 3.33 | 38.34 |
| stdev | | 28.433 | 1.095 | 2.689 | 1.446 | 0.564 | 0.667 | 5.918 | 4.768 | 36.637 |

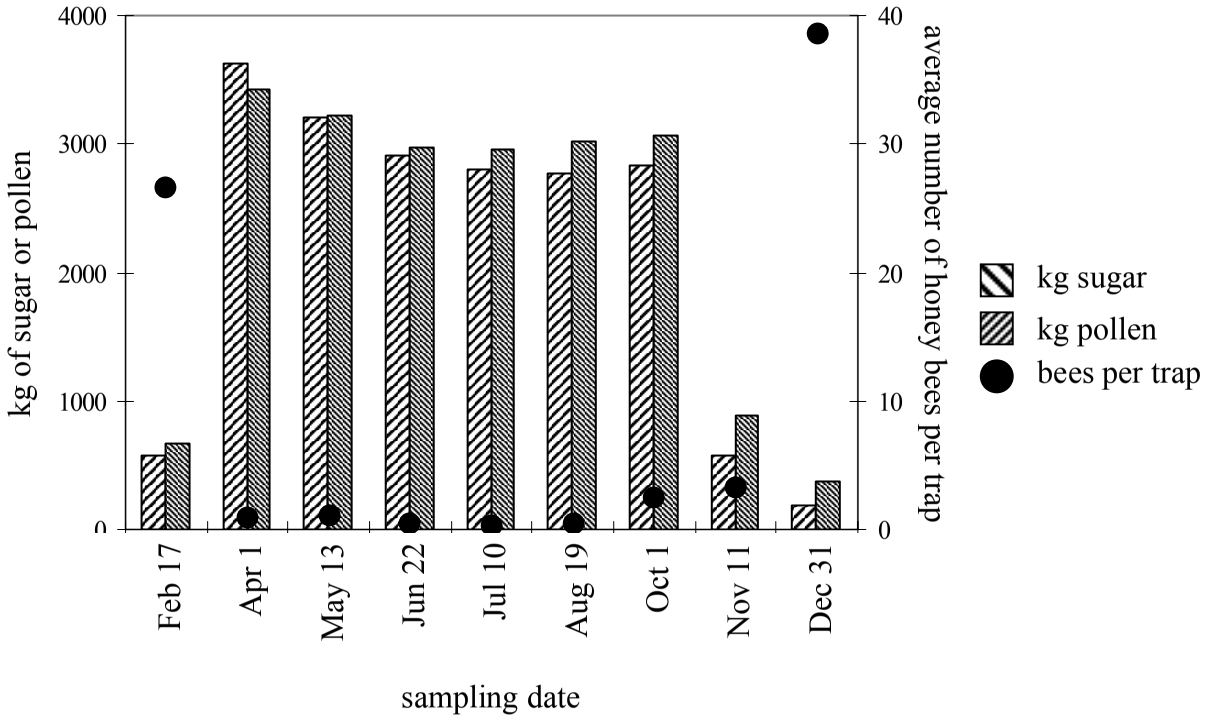



Figure 46. Mean number of honey bees collected per trap in relation to nectar and pollen availability for each sampling period. Sampling dates represent the end of a three-week sampling period.

Table 27. The results from Kruskal-Wallis (H and p-values) and Mann-Whitney *U* (U and p-values) tests comparing the number of honey bees collected per trap between habitat types, where B = brushland, G = grassland, O = live oak, and W = woodland. The habitat type with the larger number of honey bees is shown in parentheses for significant differences at the $\alpha = 0.05$ level. Sampling dates represent the end of a three-week sampling period. Mean numbers of honey bees collected per trap for each habitat type and sampling period are shown in Figure 3.

| | Kruskal-Wallis | | | Mann-Whitney *U* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | H | p-value | | B-G | B-O | B-W | G-O | G-W | O-W |
| Feb 17 | 9.987 | 0.0187 | U | 15 | 68 | 25.5 | 28.5 | 3 | 21 |
| | | | p-value | 0.2744 | 0.2826 | 0.0425 (B) | 0.8805 | 0.0203 (G) | 0.0054 (O) |
| Apr 1 | 2.334 | 0.5061 | | | | | | | |
| May 13 | 11.917 | 0.0077 | U | 12 | 36 | 42 | 29.5 | 12 | 38 |
| | | | p-value | 0.0114 (G) | 0.0016 (O) | 0.0939 | 0.9579 | 0.2542 | 0.0517 |
| Jun 22 | 0.943 | 0.8150 | | | | | | | |
| Jul 10 | 9.493 | 0.0234 | U | 10 | 72 | 42 | 9.5 | 5.5 | 66 |
| | | | p-value | 0.0565 | 0.2229 | 0.2482 | 0.0079 (G) | 0.0180 (G) | 0.8760 |
| Aug 19 | 5.940 | 0.1146 | | | | | | | |
| Oct 1 | 3.062 | 0.3821 | | | | | | | |
| Nov 11 | 8.347 | 0.0394 | U | 7 | 69 | 31 | 19 | 4 | 35.5 |

Table 27. Continued.

| | Kruskal-Wallis | | | Mann-Whitney *U* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | H | p-value | | B-G | B-O | B-W | G-O | G-W | O-W |
| Nov 11 | | | p-value | 0.0336 (G) | 0.2991 | 0.0914 | 0.2667 | 0.0227 (G) | 0.0470 (O) |
| Dec 31 | 11.769 | 0.0082 | U | 22 | 64 | 13 | 21 | 3 | 19 |
| | | | p-value | 0.8082 | 0.3030 | 0.0034 (B) | 0.4568 | 0.0201 (G) | 0.0055 (O) |

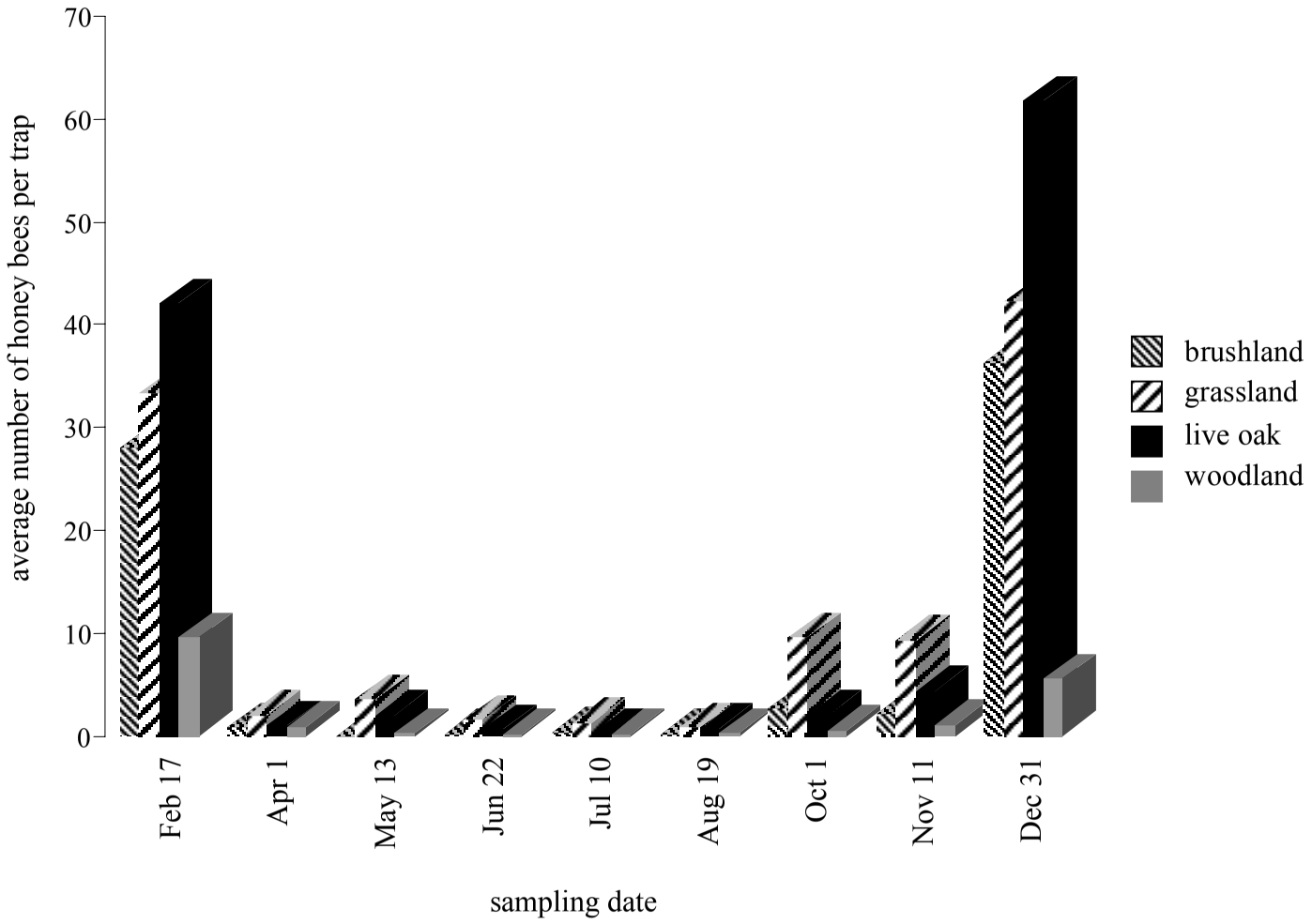



Figure 47. Mean number of honey bees collected per trap for each habitat type and sampling period. Sampling dates represent the end of a three-week sampling period.

## DISCUSSION

The honey bees collected in the traps could not recruit to the traps, so the traps provided an estimate of how many honey bees were searching for resources in the immediate area. However, honey bees recruiting to a specific resource may find another nearby one unintentionally, so not all bees collected in the traps were necessarily searching for new resources, especially when floral sources were abundant near a trap. Based on Biesmeijer and de Vries (2001), three types of foragers can find new food sources, including 1) novice bees, 2) experienced, unemployed foragers, and 3) lost recruits. Therefore, the number of honey bees collected in the traps represent these three types of foragers, as well as individuals searching for other resources, such as cavities.

The distance bees search for resources varies depending on the resource of interest. Camazine et al. (1999) recorded bees from a single swarm dancing for over 100 different natural nest sites, ranging 554 to 9520 m from the swarm. Based on engorgement and metabolic rates, the maximum flight distance is 64 km for reproductive swarms and 131 km for absconding colonies (Otis et al. 1981). Foraging distances range from a few meters to over 10000 m (Gary et al. 1972, Visscher and Seeley 1982, Schneider 1989, Schneider and McNally 1993, Waddington et al. 1994, Schneider and Hall 1997, Beekman and Ratnieks 2000). Few studies have examined water collecting behavior, but Gary et al. (1979) found that honey bees typically collect water from sources near the colony, with a mean distance of 88.9 m and a maximum of 2337 m. Honey bees may also advertise propolis sources with dances (Milum 1955), but little is known about recruitment to propolis sources. Therefore, the distance from a colony at

which a honey bee would be collected in a trap varies depending on the resource of interest.

Two swarming periods occur in southern Texas. The primary swarming period is from April through June, sometimes followed by a brief secondary swarming period between August and October (Rubink et al. 1990b, Rubink et al. 1996). Relatively few honey bees were collected during these potential swarming periods (Table 26), suggesting that most of the honey bees collected in the traps were searching for food sources.

Search rates varied throughout the year and were highest from December through February, when nectar and pollen availability were very low, suggesting the number of honey bees collected in the traps was inversely related to resource availability (Table 26, Figure 47). Seeley (1983) also found that the number of bees searching for food resources varied with resource availability, ranging from 5 to 36 % when resources were abundant and scarce, respectively. Search rates also varied between habitat types at certain times of the year (Table 27, Figure 47). Sugden et al. (1989) observed differences between riparian, brushland, and suburban habitats in honey bees collected in aerial pitfall traps during a period of nectar flow. In this study, traps in the woodland habitat contained fewer honey bees than those located in other habitats from December through February (Table 27). Estimates of nectar availability were also lower in the woodland habitat compared to the brushland, grassland, or live oak habitats (Figure 46). Therefore, honey bees may search habitats in proportion to resource availability. However, these patterns were not as clear for other time periods.

The importance of recruitment may vary depending on the distribution of resources. For example, Waddington et al. (1994) found recruitment was less important in an environment with abundant resources distributed among many small patches. Recruitment is probably more important in environments with a few high quality patches or under dearth conditions. Therefore, the number of honey bees collected in the traps may provide information about the distribution, as well as abundance, of food sources.

In conclusion, honey bees searched for resources at different rates throughout the year, with the largest number of bees collected in the traps from December through February, when nectar and pollen availability were very low. Search rates also varied between habitat types at certain times of the year. Traps in the woodland habitat contained fewer honey bees than those located in other habitats from December through February. Estimates of nectar availability were also lower in the woodland habitat compared to the brushland, grassland, or live oak habitats. Few honey bees were collected during the swarming periods, suggesting that most of the honey bees collected in the traps were searching for food sources. Therefore, the number of honey bees collected in the traps provided a qualitative estimate of food resource availability.

Detailed measurements of nectar and pollen influxes into colonies in relation to the number of honey bees collected in the traps could provide more quantitative information on resource availability that may allow for comparisons between different areas. Aerial pitfall traps could also be used to collect honey bees for genetic analysis, including monitoring for Africanized honey bees or estimating the number of colonies present in an area. Rubink et al. (1990a) suggest aerial pitfall traps may provide an

alternative estimate of population size compared to bait hives, especially in areas where natural nest sites are abundant and swarm capture rates are low. Numerous other arthropods were frequently collected in the traps, including Anthophoridae (*Ancyloscelis*, *Exomalopsis*, *Xylocopa*), Apidae (*Bombus*), Colletidae (*Colletes*), Halictidae (*Agapostemon*, *Augochlorella*, *Lasioglossum*), Megachiladae (*Megachile*), Araneae, Coleoptera, Diptera, and Lepidoptera. The traps also may be useful for examining patterns in the spatial and temporal distribution of members of these groups.

# CHAPTER VI

# CONCLUSIONS

The goal of my dissertation was to examine the ecology of feral honey bee colonies on the Welder Wildlife Refuge, including the identification of the habitat associations of the feral colonies, an evaluation of changes in the distribution and abundance of the feral colonies, and an examination of resource use by the feral colonies. More specifically, I defined the functional heterogeneity of feral honey bee habitat by identifying the suitability of different habitats for feral colonies based on the distribution and abundance of important resources (cavities, nectar, and pollen). I evaluated the distribution and abundance of feral colonies by examining nest site characteristics, population trends, and spatial and temporal patterns in cavity use. Lastly, I examined resource use by evaluating patterns in pollen collection and identifying where and when honey bees searched for resources.

The approach of defining a rule base for feral honey bees in a coastal prairie landscape based on landscape classifications of important resources proved a valuable methodology for defining the functional heterogeneity of feral honey bee habitat. The combined rankings for cavity, nectar, and pollen sources showed that the dense live oak habitat was the best overall source for cavities, pollen, and nectar. Resources appeared not to be limiting to feral honey bees in the study area, except during December and January. However, the distribution and abundance of cavity, nectar, and pollen sources varied at different spatial scales. Cavity, nectar and pollen sources were abundant within

the study area and on the Welder Wildlife Refuge, less abundant within San Patricio County, and moderately abundant within the Texas coastal bend ecoregion.

The colony densities of up to 12.5 colonies per $km^2$ observed in the study area were the highest reported in the literature for an area including both suitable and unsuitable habitat. The measured cavity attributes were similar to those reported from other areas. The time occupied and turnover indices provided useful information about cavity quality. However, none of the measured cavity attributes were correlated with the time occupied and turnover indices. Therefore, cavities appeared not to vary in their suitability for honey bees based on the measured structural and environmental attributes, but probably varied in quality based on unmeasured cavity characteristics.

Spatial patterns existed in cavity use by the feral colonies, with the colonies showing an aggregated pattern of distribution throughout the time period of this study. Colony aggregations probably resulted from the distribution of resources, especially cavities, although none of the proposed explanations could be rejected. The spatial and temporal distribution of European and Africanized colonies represented the invasion of Africanized honey bees. Two years after the arrival of Africanized bees, Africanized and European colonies were aggregated. 1997 appeared to be a transition period, with the random distribution of Africanized and European colonies. After that time, European colonies remained randomly distributed, while Africanized colonies were aggregated. Therefore, the invasion of Africanized honey bees appeared to fragment the existing European population, corresponding to a decrease in the overall number of European colonies in the study area.

The feral colonies collected a wide variety of pollen types. Anemophilous pollen types were important in the fall, while entomophilous pollen types were important for most of the year. Herbaceous plants and shrubs provided pollen during the spring and early summer, trees in mid to late summer, and herbaceous plants in the fall. The pollen sources utilized by the feral colonies also tended to be good nectar sources. Overlap in pollen use between colonies varied throughout the year. Pollen overlap was correlated with distance for some sampling periods and not others, probably due to the way colonies choose among resources and the flowering phenology in the study area.

Honey bees searched for resources at different rates throughout the year, with the largest number of bees collected in the traps from December through February, when nectar and pollen availability were very low. Search rates also varied between habitat types at certain times of the year. Traps in the woodland habitat contained fewer honey bees than those located in other habitats from December through February. Estimates of nectar availability were also lower in the woodland habitat compared to the brushland, grassland, or live oak habitats. Few honey bees were collected during the swarming periods, suggesting that most of the honey bees collected in the traps were searching for food sources. Therefore, the number of honey bees collected in the traps provided a qualitative estimate of food resource availability.

In conclusion, the Welder Wildlife Refuge provided excellent habitat for feral honey bees, supporting a high density of colonies. The colonies were aggregated within the study area, possibly due to the distribution of resources. Resources were abundant throughout the year, with the exception of December and January, when a large number

of bees searched for resources. Resources were less abundant at the county and ecoregion scales.

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# VITA

Kristen Anne Baum was born on 29 September 1972 in Waynesboro, Virginia. Kristen's permanent address is 1100 Tanbark Drive, Afton, Virginia, 22920. Kristen received her B.S. in environmental science from the College of William and Mary, Williamsburg, Virginia in May 1994, her M.S. in wildlife and fisheries sciences from Texas A&M University, College Station, Texas in May 1999, and her Ph.D. in entomology from Texas A&M University, College Station, Texas in May 2003.